ATTACHMENT 14

Human Health Effects Literature

DOES NOT CONTAIN CBI

Case report

Clustered sensitivity to fungi: anaphylactic reactions caused by ingestive allergy to yeasts

Kristiina Airola, MD, PhD; Leena Petman; and Soili Mäkinen-Kiljunen, PhD

Background: Respiratory allergy to environmental molds is relatively common, and fungal allergen–specific reactivity seems to cluster in certain persons. However, generalized reactions caused by ingested fungi have seldom been described.

Objective: To describe a mold-sensitized patient who developed multiple anaphylactic reactions after ingesting a yeast preparation widely used by the food industry as flavoring in, for example, powdered and ready-made sauces.

Methods: Skin prick tests and serum IgE tests were performed with inhalant and food allergens, including molds and yeasts, 2 pasta sauces consumed by the patient, individual sauce ingredients, and a food-quality yeast extract. Radioallergosorbent test inhibition was used for specificity studies.

Results: Skin prick and serum IgE test results were positive to several molds (*Cladosporium herbarum, Alternaria alternata, Aspergillus fumigatus*, and *Penicillium notatum*), baker's yeast (*Saccharomyces cerevisiae*), *Malassezia furfur*, and champignon and to the 2 pasta sauces, the yeast ingredient, and a food-quality yeast extract. Radioallergosorbent test inhibition studies confirmed that the sauces contain cross-reacting yeast and mold allergens.

Conclusions: This patient has a clustered sensitization to fungi characterized by allergy to environmental fungal allergens and to yeast extracts used in the food industry. Yeasts should be considered as possible ingestive allergens in mold-allergic patients.

Ann Allergy Asthma Immunol. 2006;97:294–297.

INTRODUCTION

Several fungal species are present in the outdoor and indoor environments and can induce allergic symptoms in the respiratory tract, mucous membranes, and skin.[1] Fungal allergen–specific reactivity seems to cluster in certain persons, and the term *multiple fungi sensitization* has been used to describe patients sensitized to 2 or more fungi species.[2,3]

Fungal allergen exposure generally leads to respiratory and skin symptoms, whereas generalized reactions are rare. A few cases of anaphylaxis have been described with fermented rice, some molds, and edible mushroom as allergens.[4–7] We report novel data of IgE-mediated sensitization to environmental fungal allergens associated with allergy to the ingestion of a yeast preparation commonly used by the food industry as a flavoring in, for example, pasta sauces.

CASE REPORT

A 29-year-old American woman was admitted to the Skin and Allergy Hospital, Helsinki University Central Hospital, for multiple severe anaphylactic reactions induced by ingested food. She has had pollen and animal dander allergy and asthma since childhood but was otherwise in good health. She previously experienced an anaphylactic reaction in the United States to contrast media and in 1999 to ingested beer, which she has avoided ever since. Subsequent anaphylactic reactions occurred in August 2003, September 2003, and September 2004 in Finland.

The patient was initially investigated at the Skin and Allergy Hospital in October 2003. In August 2003, ingested red wine had induced a mild anaphylactic reaction that she treated with an epinephrine autoinjector and antihistamine. In September 2003, just minutes before the allergic reaction she had a meal of industrially made spaghetti Bolognese sauce, pasta, and bread. The reaction was treated in an emergency unit with 3 subsequent doses of epinephrine, intravenous corticosteroid and theophylline, and inhaled salbutamol. Skin prick test (SPT) results were positive for soya, various nuts and seeds, food additive E163 (anthocyanin), and beer malt containing barley, but no conclusive etiologic agent was confirmed.

The next anaphylactic reaction took place in September 2004. The asthma, previously in good balance, had been labile for the past year. A few minutes before the reaction, the patient had had a meal of industrial-made olive sauce, pasta, and feta cheese. Neither physical exercise nor the use of any drug preceded this or any other anaphylactic reaction. The patient could not use her epinephrine autoinjector because of the severe allergic symptoms, including angioedema of the throat, difficulties in breathing, and near loss of consciousness; as the emergency unit reached her she was cyanotic, with a blood oxygen level of 56%, and disoriented. She was treated with 5 doses of epinephrine, intravenous corticosteroid and theophylline, inhaled ipratropium bromide, and ox-

Skin and Allergy Hospital, Helsinki University Central Hospital, Helsinki, Finland.

Received for publication December 13, 2005.
Accepted for publication in revised form March 21, 2006.

ygen. Three weeks later, she was reinvestigated at the Skin and Allergy Hospital.

METHODS

Skin Prick Tests

The SPTs were performed using commercial test solutions (ALK-Abello, Horsholm, Denmark) and 7 separate ingredients of the Bolognese sauce, including a yeast preparation provided by the manufacturer. In addition, a commercial food-quality yeast extract preparation (Sensient, Strasbourg, France) and several brands of red and white wine containing wine yeast were tested.

Serum Allergen Specific IgE and Inhibition Tests

Serum mold and yeast specific IgE levels were measured using the commercial CAP-FEIA System (Pharmacia, Uppsala, Sweden). In addition, in-house ImmunoSpot and radioallergosorbent test (RAST) methods were used to evaluate specific IgE to mold and yeast skin test extracts and to the 2 pasta sauces, individual sauce ingredients, the commercial yeast extract preparation, and the wines.[8] Native and denatured yeast allergens were evaluated in an IgE immunoblot after sodium dodecyl sulfate–polyacrylamide gel electrophoresis.

The RAST inhibition was used to appraise specific and cross-reacting allergens.[8] In brief, the patient's serum was first incubated with an extract made from the sauce mix, the 2 yeast extracts, and a yeast and mold skin test extract (*Saccharomyces cerevisiae* and *Cladosporium herbarum*). The remaining IgE-binding capacity to the respective commercial solid-phase mold and yeast antigens was subsequently measured using a specific IgE blood test (ImmunoCAP; Pharmacia).

RESULTS

The patient was atopic, as determined by positive SPT reactions to pollen and animal dander. In the same test series, a strong positive reaction to *C herbarum* was detected (wheal, 10 mm; positive histamine control, 5 mm). The patient also had a positive SPT reaction (>3 mm) to other molds tested (*Alternaria alternata, Aspergillus fumigatus,* and *Penicillium notatum*), baker's yeast (*S cerevisiae*), *Malassezia furfur*, and champignon. In addition, both pasta sauces were positive (4 mm). Of the 7 separate ingredients of the Bolognese sauce mix obtained from the manufacturer (bay leaf, garlic, oregano, paprika, pepper, rosemary, and yeast extract), only the yeast extract yielded a positive reaction (5 mm), and its strict composition was not given. Other ingredients of both sauce mixes (black olive, green olive, capers, monosodium glutamate, chili, tarragon, garden sage, basil, onion, potassium nitrate, and sodium nitrate) produced negative results. Results of SPTs with the yeast extract preparation in 20 healthy volunteers were negative.

The allergens of the yeast extract were stable, because baking, frying, microwaving, and freezing of the extract did not alter the SPT reaction (wheal, 5–7 mm). The commercial food-quality yeast extract preparation produced similar SPT results (5 mm), whereas maltodextrin present in the mix as a carrier tested negative. Red and white wines gave variable SPT reactions (3–5 mm). No controls were tested, and the reactions were interpreted as negative (nonspecific) because no specific IgE for various wine brands could be detected in the immunospot assay (data not shown).

The serum total IgE concentration was slightly elevated (127 kU/L), consistent with the atopic diathesis. The specific IgE level for *C herbarum* was 4.79 kU/L (positive cutoff limit, 0.35 kU/L), for *A alternata* was 3.52 kU/L, for *A fumigatus* was 8.56 kU/L, for *P notatum* was 3.71 kU/L, and for *S cerevisiae* was 2.74 kU/L. Immunospot studies showed specific binding of the patient's serum IgE to fungal allergens, both sauce mixes, and the yeast extract preparations immobilized on a nitrocellulose membrane (Fig 1). Yeast extract showed specific IgE binding even after baking, frying, microwaving, and freezing (data not shown).

In-house RAST with the commercial yeast extract showed high IgE binding of the patient's serum (8% of the total activity) compared with binding of the nonatopic control serum and the atopic control serum of pooled timothy IgE–positive sera (0.3% binding each). In immunoblot, no distinct IgE binding was detected after sodium dodecyl sulfate–polyacrylamide gel electrophoresis with native or denatured yeast

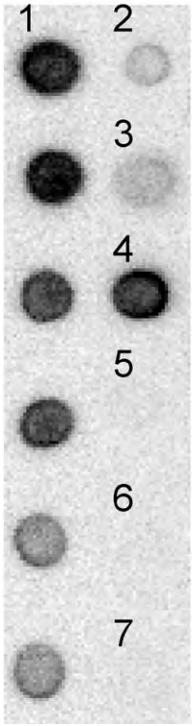


Figure 1. Autoradiogram of IgE binding of the patient's serum to yeasts and molds in immunospot. 1 indicates yeast extract (1:4 wt/vol) and two 2-fold dilutions in duplicate; 2, *Alternaria alternata*; 3, *Aspergillus fumigatus*; 4, *Cladosporium herbarum*; 5, *Penicillium notatum*; 6, *Saccharomyces cerevisiae*; and 7, *Malassezia furfur*. Bound IgE was detected by using iodine 125–labeled anti–human IgE antibody.

allergen extracts. At most, faint staining was seen at the start line and at the lower solvent front. This poor response is probably due to glycoproteins present in yeast extracts.[9]

In RAST inhibition, preincubation of the patient's serum with the Bolognese sauce mix and the 2 yeast extracts significantly reduced IgE binding to yeast (*S cerevisiae*) and mold (*C herbarum*) antigens (45%–49% inhibition with the sauce mix and 70%–84% inhibition with the yeast extracts) (Fig 2). This indicates that the patient has cross-reacting IgE for the fungal antigens present in the food mix and the yeast extracts.

DISCUSSION

The prevalence of allergy to environmental fungi, particularly *C herbarum* and *A alternata*, is estimated to be 3% to 20% in the general population.[10,11] A recent survey[12] from the United States reported a prevalence of 12.9% for *Alternaria*. Fungal allergen–specific reactivity seems to cluster in certain persons, leading generally to respiratory and skin symptoms and only seldom to food-allergic reactions.[4–7] Despite the high prevalence and clustering effect of fungal allergy, ingested yeast species have not previously been recognized as potential allergens.

The patient described herein is an American woman with labile asthma. She had experienced multiple severe anaphylactic reactions after ingesting food or drinks. The initial intense SPT reaction to *C herbarum* led us to suspect fungal allergy, and the subsequent investigations with the yeast-containing pasta sauces and pure yeast samples confirmed this suspicion.

The only ingredient of the pasta sauce to which the patient showed a positive SPT response and specific IgE was the yeast extract. The in vitro IgE response was unambiguous in immunospot and RAST studies, but in immunoblotting, only faint binding at the start and at the lower front could be detected. The reason for this is not clear, but allergen proteins may appear in aggregates and remain on the start line. Alternatively, they may be split into small peptides that go off electrophoresis gel or penetrate the nitrocellulose sheet when transferred from the gel. The detailed content of the yeast preparations was not available, but at least the commercial extract contained maltodextrin, which may hamper electrophoresis.

All the severe reactions took place in autumn, when the concentration of molds in the air is generally high in Finland. The patient had experienced considerable mental stress during the previous year because of a chronic disease of her child, and this may have contributed to the worsening of the previously stable asthma. The inhalative exposure to mold aeroallergens in autumn may have increased the patient's sensitivity to the yeast species ingested. During the investigations, she had had 3 more minor anaphylactic reactions after the ingestion of yeast species (between November 2004 and January 2005) that she successfully treated herself with an epinephrine autoinjector and peroral corticosteroid. Because of the repeated severe anaphylactic reactions, a food

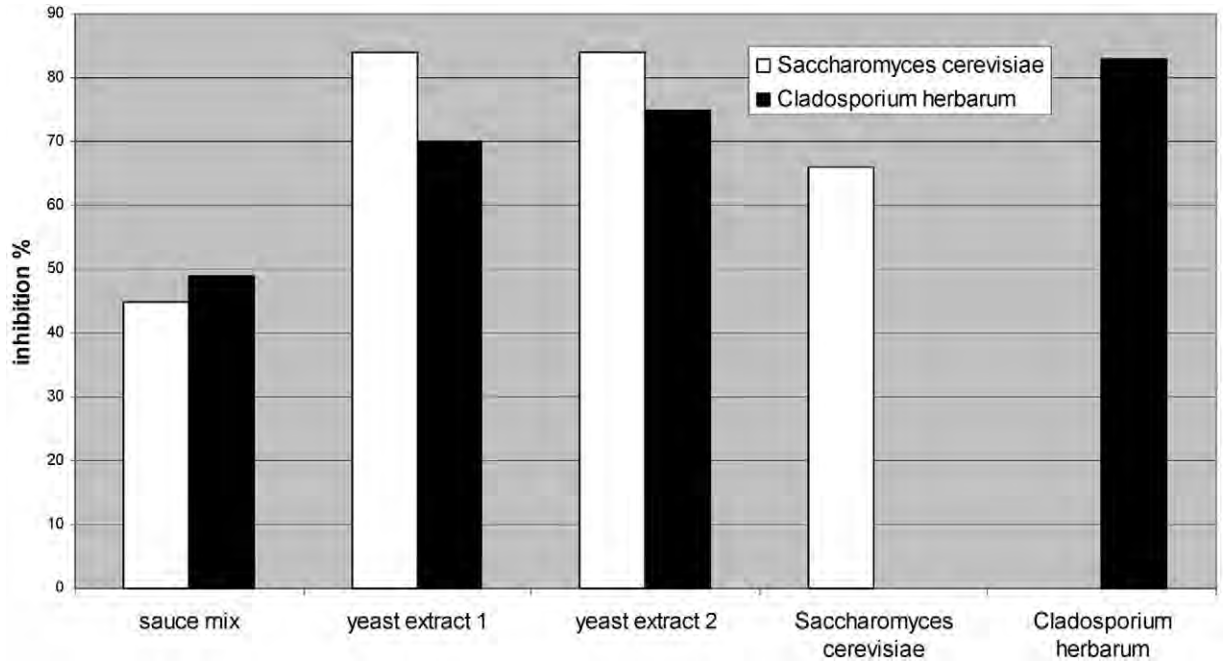


Figure 2. Inhibition of the binding of the patient's serum IgE to mold allergens. In radioallergosorbent test inhibition, the patient's serum was first incubated with the Bolognese sauce mix, the 2 yeast extracts, and control antigens (*Saccharomyces cerevisiae* or *Cladosporium herbarum* skin test extract) before probing with the solid-phase antigens (*Saccharomyces* or *Cladosporium* ImmunoCAP).

challenge test was not justifiable. However, an elimination diet has been effective: the patient is now doing fine (at 1 year) by carefully avoiding all foods containing industrial yeast extracts. In fact, the strict avoidance has resulted in a reduction in her yeast specific IgE binding from 8% to 4% in 10 months.

It is of interest that the patient can freely eat bakery products but experiences severe allergic reactions after eating sauces and other foods containing yeast as a flavoring. This can be explained by differences in yeast species used in the extracts and possibly also by chemical modification required in the extraction of the yeast. The extracts typically contain species such as *S cerevisiae* (baker's yeast or brewer's yeast) or *Saccharomyces pastorianus* (lager yeast). *Saccharomyces* species show high variability at the genotypic and phenotypic levels.[13] Our patient has been avoiding beer since the first anaphylactic reaction in 1999, and our commercial yeast extract used for IgE assays is derived from *S pastorianus*, suggesting that this species is the main allergen for the patient. Based on the data presented herein, we suggest that yeasts should be considered as possible ingestive allergens in persons sensitized to fungi.

REFERENCES

1. Kurup VP, Shen H-D, Vijay H. Immunobiology of fungal allergens. *Int Arch Allergy Immunol*. 2002;129:181–188.
2. Potter PC, Juritz J, Little F, et al. Clustering of fungal allergen-specific IgE antibody responses in allergic subjects. *Ann Allergy*. 1991;66:149–153.
3. Mari A, Schneider P, Wally V, et al. Sensitization to fungi: epidemiology, comparative skin tests, and IgE reactivity of fungal extracts. *Clin Exp Allergy*. 2003;33:1429–1438.
4. Wigger-Alberti W, Bauer A, Hipler U-C, Elsner P. Anaphylaxis due to *Monascus purpureus*–fermented rice (red yeast rice). *Allergy*. 1999;54:1330–1331.
5. Bennett AT, Collins KA. An unusual case of anaphylaxis. *Am J Forensic Med Pathol*. 2001;22:292–295.
6. Hoff M, Trueb RM, Ballmer-Weber BK, Vieth S, Wüthrich B. Immediate-type hypersensitivity reaction to ingestion of mycoprotein (Quorn) in a patient allergic to molds caused by acidic ribosomal protein P2. *J Allergy Clin Immunol*. 2003;111:1106–1110.
7. Torricelli R, Johansson SGO, Wutrich B. Ingestive and inhalative allergy to the mushroom *Bolutus edulis*. *Allergy*. 1997;52:747–751.
8. Mäkinen-Kiljunen S. Banana allergy in patients with an immediate type hypersensitivity to latex: characterization of cross-reacting antibodies and allergens. *J Allergy Clin Immunol*. 1994;93:990–996.
9. Leino M, Reijula K, Mäkinen-Kiljunen S, Haahtela T, Makela MJ, Alenius H. *Cladosporium herbarum* and *Pityrosporum ovale* allergen extracts share cross-reacting glycoproteins. *Int Arch Allergy Immunol*. 2006;140:30–35.
10. D'Amato G, Chatzigeorgiou G, Corsico R, et al. Evaluation of the prevalence of skin prick test positivity to *Alternaria* and *Cladosporium* in patients with suspected respiratory allergy: a European multicenter study promoted by the Subcommittee on Aerobiology and Environmental Aspects of Inhalant Allergens of the European Academy of Allergology and Clinical Immunology. *Allergy*. 1997;52:711–716.
11. Reijula K, Leino M, Mussalo-Rauhamaa H, et al. IgE-mediated allergy to fungal allergens in Finland with special reference to *Alternaria alternata* and *Cladosporium herbarum*. *Ann Allergy Asthma Immunol*. 2003;91:280–287.
12. Arbes SJ, Gergen PJ, Elliott L, Zeldin DC. Prevalence of positive skin test responses to 10 common allergens in the US population: results from the Third National Health and Nutrition Examination Survey. *J Allergy Clin Immunol*. 2005;116:377–383.
13. Rainieri S, Zambonelli C, Kaneko Y. *Saccharomyces sensu stricto*: systematics, genetic diversity and evolution. *J Biosci Bioeng*. 2003;96:1–9.

Requests for reprints should be addressed to:
Kristiina Airola, MD, PhD
Skin and Allergy Hospital
Helsinki University Central Hospital
POB 160
00029 HUS, Finland
E-mail: kristiina.airola@hus.fi

is the persisting and increasing edema over weeks or months without the subsidence of swelling seen in common angioedema with other causes.

REFERENCES

1. Castellani A. Elephantiasis nostras. J Trop Med Hyg 1934; 37:257-64.
2. Castellani A. Researches on elephantiasis nostras and elephantiasis tropica with special regard to their initial stage of recurring lymphangitis. J Trop Med Hyg 1969;72:89-97.
3. Oomen AP. A reconsideration of the problem of elephantiasis. Trop Geogr Med 1969;21:225-35.
4. Sheldon JM, Lovell RG, Mathews KP, eds. Dermatologic aspects of allergy practice. In: A manual of clinical allergy. Philadelphia: WB Saunders Company, 1967:226-67.
5. Hollander DI, Halwig JM, McKinney P, Patterson R. Elephantiasis nostras 1984. J ALLERGY CLIN IMMUNOL 1985;75:450-51.
6. Cranberg JA, Patterson R, Caro WA. Angioedema, elephantiasis nostras, and cheilitis granulomatosa. Allergy Proc 1990;11:79-82.
7. Beninson J. Successful treatment of elephantiasis nostras of the lip. Angiology 1971;22:448-55.

Baker's asthma caused by *Saccharomyces cerevisiae* in dry powder form

Juan Belchi-Hernandez, MD, Ana Mora-Gonzalez, MD, and Josefa Iniesta-Perez *Murcia and Elche, Spain*

Bakers' asthma is a well-defined disease. It can be caused by various antigens: flours and bran, yeast, baking additives, saprophytic molds, and storage mites.[1-3] It is well known that hypersensitivity to flour represents a significant proportion of occupational allergies.[4, 5] However, in recent years many more substances used in baked goods and pastry have been reported as causes of allergy.

In 1713 Ramazzini, in *De Morbis Artificum Diatriba,* stated that work environments and products were factors responsible for disease. We believe it is important to be familiar with the workplace and existing dust products and the procedures for handling such products to be able to establish a correct etiologic diagnosis.

We report a case of bakers' asthma caused by an antigen that is always present in the baking industry but does not easily affect the airways and rarely sensitizes workers.

CASE REPORT

A 48-year-old man who was a nonsmoker came to our attention with repeated episodes of hydrorrhea, sneezing, nasal obstruction, wheezing, spasmodic cough, and dyspnea. The symptoms would always appear 1 to 2 hours after starting work. They first appeared a year ago on weekdays and improved on Sundays (the patient's rest day). The symptoms were initially mild but became progressively worse until continuous medical treatment was required. During short holiday periods he was free of symptoms and did not need bronchodilators. Results of an allergy test were negative, and the patient was diagnosed as having rhinitis and intrinsic asthma. Treatment was started with budesonide and salbutamol, but the symptoms were not fully controlled.

Abbreviations used
PEFR: Peak expiratory flow rate
SC: *Saccharomyces cerevisiae*

The bakery is approximately 90 m^2, well-ventilated, and not near the patient's home. There are two other workers, besides the patient, who remain free of symptoms. No changes have been implemented in the last few years. Contamination by molds and mites not used in the skin tests was ruled out by an optical microscopy study of different dust samples.[6] The bread ingredients are as follows: water, salt, wheat flour and bran, dry powder dehydrated yeast, and baking additives containing exogenous enzymes (amylases, glucoamylase, and lipoxygenase), antioxidant agents (potassium bromate, azodicarbonamide, ascorbic acid, and soybean flour), and emulsifiers (soybean lecithin). When the flour, yeast, and additives are mixed, a dusty environment is created, which decreases once cold water is added and kneading is started.

Three years ago as a result of storage problems, the conventional yeast (nondehydrated, 70% to 75% humidity and requiring a temperature of 4° C for proper

From the Department of Allergy, J. Mª Morales Meseguer Universitary Hospital, Murcia; and the Department of Biology, High School Carrús, Elche.
Reprint requests: Juan Belchi-Hernandez, MD, c/ Torres, no. 11, 1ª Esc. 7° C, 30005 Murcia, Spain.
J ALLERGY CLIN IMMUNOL 1996;97:131-4.

0091-6749/96 $5.00 + 0 **1/54/67524**

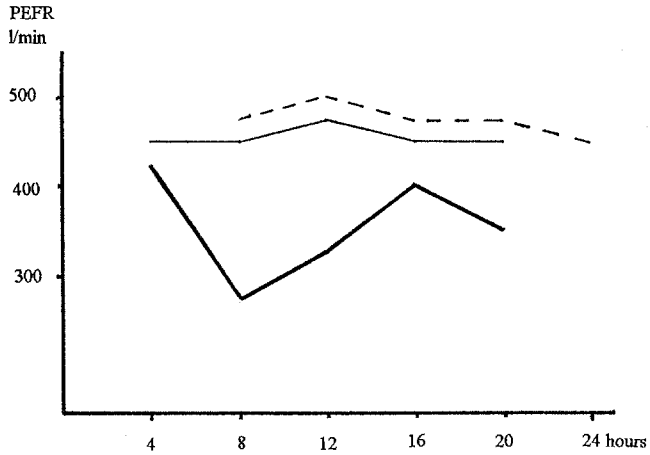


FIG. 1. Bronchial response patterns during PEFR monitoring: (▬▬) working with dry yeast, (——) working with wet yeast, (----) on vacation.

storage) was replaced with dehydrated yeast (dry powder, 8% humidity and storable at ambient temperature).

METHODS

We used dehydrated yeast in dry powder form, conventional wet yeast, and a commercial mixture of baking additives. For the preparation of the crude extracts, proteins were extracted by incubation of dry yeast, wet yeast, and baking additives in phosphate-buffered saline (pH 7.3) at 1:10 wt/vol. The resulting suspensions were stirred for 6 hours at 4° C and then centrifuged. The supernatants were passed through Whatman 2 filter paper (Whatman Inc., Clifton, N.J.) and then through a 0.22 μm Millipore filter (Millipore Corp., Bedford, Mass.) for sterilization. Five 10-fold dilutions (1:1, 1:10, 1:100, 1:1000, and 1:10,000) were made for the skin prick tests and to enable selection of a safe initial dose for the specific bronchial provocation test.

Skin prick tests were performed on the volar side of the forearm with a prick lancetter (DHS; Bayer, Leverkusen Germany) and with the extracts described previously. A battery of inhalant allergens including *Dermatophagoides species,* storage mites, molds (*Alternaria, Cladosporium, Aspergillus, Fusarium,* and *Rhizopus* species), pollens from our geographic area (*Artemisia vulgaris, Chenopodium album, Salsola kali, Olea europea, Parietaria judaica, Plantago lanceolata,* and grasses), wheat, barley and soybean flours (Abello S.A., Madrid, Spain), and α-amylase (Ifidesa- Aristegui, Bilbao, Spain) were tested. Histamine phosphate, 10 mg/ml, and phosphate-buffered saline were used as positive and negative controls. A positive response was defined as a wheal with a mean diameter greater than 5 mm in the presence of a negative response to the diluent. Four exposed asymptomatic bakers and eight nonexposed asthmatic patients were tested to determine that yeast and baking additive extracts are not irritating. Specific IgE was measured by enzyme immunoassay (CAP system, Pharmacia Diagnostics, Uppsala, Sweden).

Baseline peak expiratory flow rate (PEFR) measurements were obtained with a mini Wright peak flow meter (Ferraris Medical, Inc., Holland, N.Y.). The patient was asked to monitor his own PEFR every 4 hours for 1 week during work time and again for 1 week when on holiday. He was to do this three times on each occasion, and the readings were to be within 5% of each other. He was also asked to record his symptoms and therapeutic requirements during this time. After 7 days away from the bakery, the patient had another PEFR monitoring performed at work, but with the dry powder yeast substituted for conventional wet yeast. The bronchial response was considered positive when the PEFR was greater than or equal to 25%. The patient had not received any drug therapy in the month before the test.

The nonspecific bronchial provocation test was performed with methacholine to determine the presence of bronchial hyperreactivity. Methacholine was administered with a Devilbiss 646 nebulizer (Devilbiss Healthcare, Inc., Somerset, Pa.) according to the method described by M. Chatham. The specific bronchial provocation test was performed with a *Saccharomyces cerevisiae* (*SC*) extract to confirm or rule out that the bronchial manifestation was due to sensitization to this allergen. This was done when the patient was not at the bakery while he was free of symptoms and not receiving any medication that could affect the results. The extract was administered with a Devilbiss 646 nebulizer at a flow rate of 0.28 ml/min and inhaled by tidal breathing for 2 minutes. We started the test with a 10^{-4} diluted dry yeast extract, which in the skin test produced a negative result. Forced vital capacity and FEV_1 were measured at 5, 10, 15, 20, 30, and 60 minutes and hourly thereafter for the next 12 hours. A week later, we performed another bronchial provocation test with a baking additive extract, starting with a 10^{-3} dilution. A positive response was defined as a decrease in FEV_1 greater than or equal to 20%.

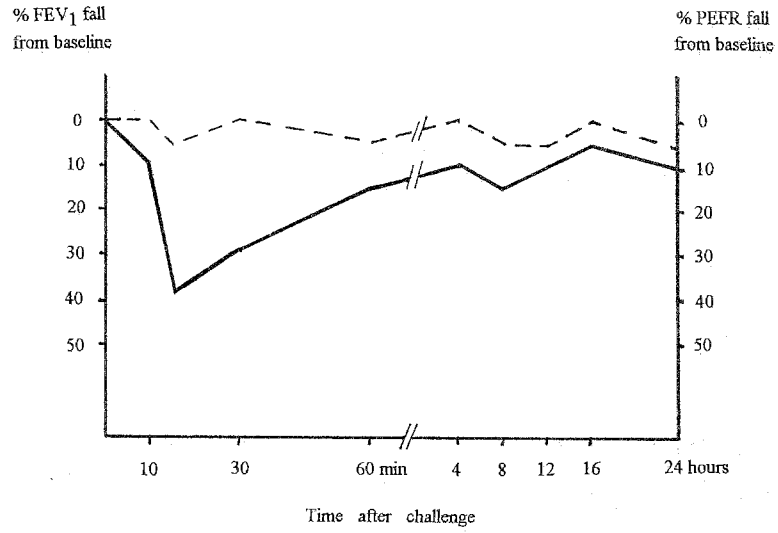


FIG. 2. Bronchial response after challenge with (----) baking additives and (▬) *SC* extract 10^{-3} wt/vol.

RESULTS

With optical microscopy we found no other contaminants that might be responsible for symptoms. The patient had negative skin test results for the battery of pneumoallergens and the baking additive extract. The skin test result was negative with 10^{-4} and 10^{-3} dilutions of the *SC* extract; with a 10^{-2} dilution it was positive for both extracts (dry and wet), with wheals of 7 mm and 9 mm, respectively.

Significant CAP binding to *SC* was demonstrated with 7.8 PRU/ml. The rest of the antigens tested did not show specific IgE.

The baseline PEFR values were within normal limits (>80% of the theoretic values). On the patient's workdays the PEFR measurements showed significant decreases from baseline values (>25%), and variability was more than 20%. During the time he was away from work, PEFR values did not fall more than 20%, and variability was less than 10%. When the patient began using conventional wet yeast, PEFR values did not decrease significantly, and variability was less than 10% (Fig. 1).

The methacholine challenge revealed bronchial hyperresponsiveness with a PD_{20} FEV_1 value of 12 accumulative breath units. No response was observed to the bronchial provocation test with baking additives. Inhalation of the 10^{-4} dilution of *SC* showed no significant changes in FEV_1 (a 6% decrease); inhalation of the 10^{-3} dilution produced a 38% drop in FEV_1, with the patient experiencing shortness of breath and wheezing. No late reactions were noted (Fig. 2).

After being diagnosed with occupational asthma caused by sensitization to *SC,* the patient began to use conventional wet yeast and carried on normal work activity without symptoms. Exercise-induced asthma persisted for a few months. He does not currently require treatment, and pulmonary function is within normal limits.

DISCUSSION

Various antigenic substances that have been reported as causes of rhinitis or occupational asthma can be found in a bakery; some of them can cause sensitization both in the home and at work. At first, considerable importance was assigned to flour proteins as sensitizing agents of the airways, with differences found between these and the proteins that cause food allergy. In recent years, baking additives have been considered an important cause of bakers' asthma,[7-10] including fermentation accelerators (enzymes), antioxidant and whitening agents (soybean flour, potassium bromate), and emulsifiers (soybean lecithin). The enzymes obtained from *Aspergillus oryzae* (α-amylase, β-amylase, and glucoamylase)[11] and soybean flour (lipoxygenase)[12, 13] are currently regarded as powerful allergens. We must not forget that these enzymes are active in wheat grain, barley, and *SC* yeast,[11] which suggests that the allergy in our patient could be due to the endogenous α-amylase in wheat. However, the qualitative and quantitative structure of these enzymes is different from that of the enzymes from *Aspergillus oryzae;* furthermore, only α-amylase from the soluble fraction of wheat grain has been reported to be allergenic.[14] The skin

prick test results were negative for flours and baking additives, and the bronchial provocation test results were negative for baking additives. This leads us to regard other *SC* proteins (e.g., enolase, peroxidase, ascorbatoxidase) as important allergenic agents,[15] some of which (enolase) have been implicated in the presence of cross-reactivity to *Candida* species.

The use of *SC* as a ferment dates to 2500 BC during the time of the Roman Empire. It has been used, practically unchanged, until the present. However, it is uncommon to find publications on sensitization to this yeast. This enzyme is always used in a cold-water solution, which is why it is difficult for a dusty environment to be formed. In contrast, flours and baking additives always form dusty environments. As a result of technical progress, yeast can now be preserved in dehydrated form and does not require special storage conditions. When *SC* yeast is used in powder form, as is the case with our patient, a dusty environment is created; therefore, we do not find it uncommon for exposed workers to be sensitized.

We know that occupational asthma in bakers is nothing new; however, the rare sensitization to such a common antigen in a bakery is indeed exceptional. It is important to be familiar with the workplace and with the procedures and materials used in order to establish a correct etiologic diagnosis.

In conclusion, our results suggest that occupational asthma caused by *SC* is uncommon in bakers despite the frequent use of this yeast. We believe it is important to be familiar with the work activity and substances to which the worker is exposed in order to establish an etiologic diagnosis of occupational asthma. It would therefore be useful to include an *SC* antigen when testing bakers with respiratory symptoms who are not sensitized to wheat grain components or enzyme additives.

REFERENCES

1. Sutton R, Skerritt JH, Baldo BA, Wrigley CW. The diversity of allergens involved in bakers' asthma. Clin Allergy 1984;14:93-107.
2. Baur X, Sauer W, Weis W. Baking additives as new allergens in baker's asthma. Respiration 1988;54:70-2.
3. Sohmen R, Rosenau C, Wittemann B, Radant S, Grieshaber R. Characterization of aeromicrobiological working conditions in a bakery. Allergologie 1993;16:248-51.
4. Armentia A, Martin-Santos JM, Quintero A, Fernandez A, Barber D. Bakers' asthma: prevalence and evaluation of immunotherapy with a wheat flour extract. Ann Allergy 1990;65:265-72.
5. Block G, Tse KS, Kijek K, Chan H. Baker's asthma. Studies of the cross-antigenicity between different cereal grains. Clin Allergy 1984;14:177-85.
6. Hocking AD, Pitt JJ. Dichloran glycerol medium for enumeration of xerophylic fungi from low moisture foods. Appl Environ Microbiol 1980;39:488-92.
7. Wuthrich B, Baur X. Baking ingredients, especially alpha-amylase, as occupational inhalation allergens in the baking industry. Schweiz Med Wochenschr 1990;120:446-8.
8. Wuthrich B, Baur X. Alpha-amylase and other antigens in the baking industry. Schweiz Med Wochenschr 1990;120: 448-50.
9. Losada E, Hinojosa M, Moneo I, Dominguez J. Occupational asthma caused by cellulasa. J ALLERGY CLIN IMMUNOL 1986;77:635-9.
10. Blanco Carmona JG, Juste Picon S, Garces Sotillos M. Occupational asthma in bakeries caused by sensitivity to alpha-amylase. Allergy 1991;46:244-6.
11. Sandiford CP, Tee RD, Newman Taylor AJ. The role of cereal and fungal amylases in cereal flour hypersensitivity. Clin Exp Allergy 1994;24:549-57.
12. Bush R, Schroeckenstein D, Meier-Davis S, Balmes J, Rempel D. Soybean flour asthma: detection of allergens by immunoblotting. J ALLERGY CLIN IMMUNOL 1988;82:251-5.
13. Lavaud F, Pardu D, Prevost A, Vallerand H, Cossart C. Baker's asthma related to soybean lecithin exposure. Allergy 1994;49:159-62.
14. Baur X, Sander I, Jansen A, Czuppon AB. Can amylases involved in bakery production be regarded as allergens? Schweiz Med Worchenschr 1994;124:846-51.
15. Baldo BA, Baken RS. Inhalant allergies to fungi: reactions to bakers' yeast and identification of bakers' yeast enolase as an important allergen. Int Arch Allergy Appl Immunol 1988;86:201-8.

BAKER'S ASTHMA

Jonas Brisman

Occup Environ Med 2002;**59**:498–502

Baker's asthma is one of the most common forms of occupational asthma. The increasing knowledge in exposure–response relations accumulated in recent years is important in the understanding of baker's asthma. This development has made scientifically based prevention feasible today and baker's asthma should not be regarded as an inevitable occurrence any more.

In 1700 Bernardo Ramazzini described respiratory symptoms among bakers caused by exposure to flour dust. However, there are anecdotal references from antiquity describing how Roman slaves working in bakeries protected themselves by using cloth as a primitive respirator to cover their faces because their breathing suffered from inhaling flour.

CLINICAL PICTURE

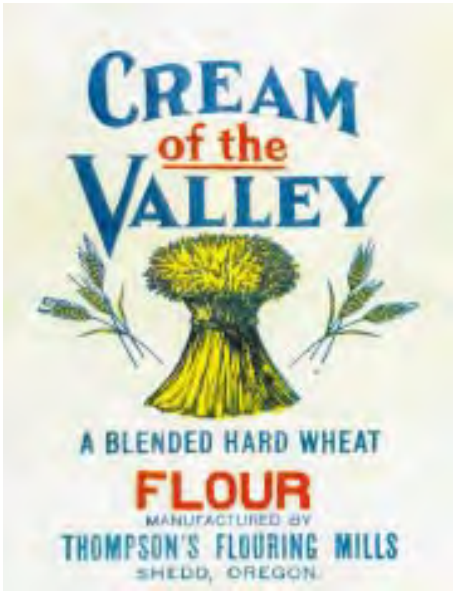
Case reports from the beginning of the 20th century established the concept of baker's asthma as an allergic disease because of the observed combination of positive skin tests to flour extracts and respiratory symptoms suggestive of asthma. The aetiological role of sensitisation to flour in these cases was confirmed by bronchial challenge tests. Rhinitis is very common and usually precedes asthma. Conjunctivitis and skin symptoms may also occur. The baker is often atopic by skin or IgE tests. Symptoms develop after a latency period of months or years, even decades. Initially there is often a clear temporal relation between symptoms and periods of bakery work. Over time, respiratory symptoms may cease to resolve during time off from the bakery. Sensitisation to flour is traditionally often regarded as a prerequisite for the diagnosis of baker's asthma. Although the prognosis of baker's asthma is not reported in the literature, it is usually presumed that symptoms resolve if exposure to offending allergens is stopped.

EPIDEMIOLOGY

From the 1930s onward there was a number of cross sectional studies surveying populations of bakers, unfortunately many of them uncontrolled—that is, without comparing the bakers with controls. These studies varied considerably in the description of symptoms, and in the definitions of asthma and sensitisation. Also exposure to bakery dust varied across the studies. Although epidemiologically crude by today's standards, they showed that bakers have more lower respiratory tract symptoms, sometimes labelled as asthma and considered as "normal", but also nasal symptoms, indicating baker's rhinitis.[1] Positive skin tests to flour were found not only among those with asthmatic symptoms but also among bakers with rhinitis or even among those without symptoms ("latent allergy"). The presence of flour allergy was usually included in the definition of Baker's asthma in the clinical setting. The earlier findings from the case series of an association between baker's asthma and atopy were corroborated in the cross sectional studies.

There are a few longitudinal studies estimating the incidence of respiratory symptoms and sensitisation to bakery allergens. Gadborg studied Danish bakers and published his results in 1956. He made a follow up of 487 out of 500 randomly selected bakers after 5–6 years. The incidence rate for sensitisation to flour was about 5.5 cases per 1000 person years, and for baker's asthma (symptoms and sensitisation) about 1.5. An often cited German study of bakers' apprentices by Herxheimer showed a cumulative incidence for sensitisation of 19% and 7% for respiratory symptoms after three years. As only one third of the original cohort were studied at that time point, the interpretation of the results is difficult. A Swedish retrospective study of trainee bakers showed male incidence rates for asthma of 3.0 cases per 1000 person-years (referents 0.9–1.9), and for rhinitis 29.4 cases per 1000 person-years (referents 10.1–11.1).[2 3] A cohort of 300 newly employed UK bakers and millers was followed for a maximum of seven years.[4] The incidence rates of work related chest symptoms was 41 per 1000 person-years, of work related eyes/nose symptoms 118 per 1000 person-years, of sensitisation to flour 22 per 1000 person-years, and of sensitisation to the enzyme fungal α amylase 25 per 1000 person-years. The incidence of work related chest symptoms and a positive skin prick test to flour or fungal α amylase was about 10 per 1000 person-years.

Correspondence to:
Dr Jonas Brisman, Department of Occupational and Environmental Medicine, Sahlgrenska University Hospital, S:t Sigfridsgatan 85, SE-412 66 Göteborg, Sweden;
jonas.brisman@ymk.gu.se



Register based studies

Baker's asthma is one of the most frequently reported forms of occupational asthma in several countries. The annual incidence in the UK was estimated to be 290–450 cases per million according to the SWORD and SHIELD schemes during 1989 to 1994. The corresponding figure for Sweden was 800 in 1984-86 and for Finland 4000 in 1990. The differences between these figures can be accounted for by quite different reporting systems and possibly also differences in exposure. A small US asthma mortality study from Chicago, conducted from 1980 to 1988 and involving individuals aged 20–35 years, showed bakers to have almost nine times the age and race adjusted mortality rate of the city's general population. A British study found no increased respiratory mortality.

ALLERGENS

Another line of development in studies on baker's asthma was to explore what agents in flour and other components in bakery dust induced the formation of IgE antibodies.[5]

Flours

The specific IgE antibodies most often found in Baker's asthma or rhinitis are against cereal flours such as wheat, rye or barley (table 1). These species are taxonomically closely related and there is strong cross antigenicity between them. There are many flour proteins with allergenic capacity; as many as 40 were described in wheat, of which 20 crossreacted with rye.[6 7] Grass is also taxonomically related to cereals, and cosensitisation and cross reactivity between cereal flours and grass has been discussed in baker's asthma. Other, non-cereal flours such as soy and buckwheat were also reported as sensitisers in bakeries and related businesses.

Enzymes

Since the 1970s a variety of enzymes can be added to flour in order to enhance the baking process. Although enzymes are used in minute quantities (typically mg/kg flour), they can cause sensitisation and baker's asthma (table 1). The most common enzyme is α amylase of fungal origin.[8] The use of α amylase varies between countries and bakeries—in some enterprises the α amylase is routinely added to the flour, in others it is used for some products only, and in some it is not used at all. The sensitisation rates for fungal α amylase vary across studies and depend on the amounts of amylase used in the different study populations.

Table 1 Allergens associated with baker's asthma and rhinitis

► Cereal flours	Wheat
	Rye
	Barley
	Hops
	Rice
	Maize
► Non-cereal flours	
	Buckwheat
	Soybean flour
► Additives	
Enzymes	α Amylase
	Cellulase
	Xylanase
	Papain, other proteases
	Glucose oxidase
Nuts	Almonds, hazelnuts
Colour	Carmine red
Spices	
► Egg powder	
► Milk powder	
► Insects	Flour beetle (*Tribolum confusum*)
	Flour moth (*Ephestia kuehnilla*)
	Cockroach (*Blatella* spp)
	Granary weevil (*Sitophilus granarius*)
► Moulds	*Alternaria, Aspergillus*
► Sesame seeds	

Other allergens

A bakery is a complex environment with a multitude of potential sensitisers, and there are case reports of baker's asthma caused by moulds, yeast, eggs, sesame seeds, nuts, and insects, for example (table 1). The occurrence of sensitisation to these allergens is less well known than those cases caused by cereal flours or enzymes, and seems to be of marginal importance to the burden of disease in bakers. However, they should be kept in mind in the clinical setting if no sensitisation to common bakery allergens is found. Storage mites have been proposed as a bakery allergen but were refuted since sensitisation rates were similar among bakers and the general population.

EXPOSURE

Knowledge about the exposure to flour dust and other allergens in bakeries is of fundamental importance when analysing the risk for asthma. Inhalation of dust as well as allergens from wheat flour and α amylase has been measured and the exposure estimated. Bakers were grouped in task groups and factors affecting the variability in the exposure were studied.[9] For the highest dust exposed task group it was shown that much of the flour dust exposure was caused by high peaks of short duration (minutes). This systematic approach provided much more detailed knowledge on the mean exposures and the variation in the different job tasks in bakeries. Together with individual information on health effects among the exposed bakers it proved to be a powerful tool for performing studies of exposure–response relations.

It was also found that a substantial proportion of flour dust particles had an aerodynamic diameter of 10 μm or more. The respirable fraction amounted to about 10% and the thoracic fraction about 40% of the total particle mass.

Table 2 Percentage of bakers with work related symptoms, positive skin prick tests (SPT) or radioallergosorbert test (RAST) to flour or any bakery allergen and the corresponding dust measurements

	Symptoms (%)		Pos SPT or RAST			Dust from personal samples	
n	Eyes/nose	Chest	Flour	Any	n	GM range (mg/m³)	Reference
133	0*		NR	NR	133	0.2–1.8	Hartmann 1986
139	11*		NR	NR	139	1.0–4.4	
42	17*		NR	NR	42	3.2–19.8	
183	13	9	5	28	32	0.01–3.0	Musk 1989
96	30	17	5	35	47	1.7–11.0	
104	11	5	2	17	205	< 1	Cullinan, Nieuwenhuijsen 1994
90	15	3	6	25	191	1–5	
62	31	11	5	30	99	>5	
117	15*		4	NR	151	0.5 (mean)	Houba 1998
107	23*		8	NR	120	0.8 (mean)	
122	29*		14	NR	178	2.4 (mean)	

*Work related symptoms from the eyes or nose and chest.
GM, geometric mean; NR, not reported.

EXPOSURE–RESPONSE RELATIONS

There are at least four published exposure–response relation studies performed in cross sectional data from Switzerland, the UK, and the Netherlands (table 2). They all showed positive exposure–response relations—that is, higher prevalence of chest and/or nasal symptoms by higher exposure to bakery dust. There were also positive exposure–response relations for sensitisation to flour, usually wheat.

Two cross sectional studies from the Netherlands and the UK showed positive exposure–response relations for the rate of sensitisation to fungal α amylase by exposure to that enzyme.

Those rather remarkably consistent results in cross sectional materials were recently corroborated by two longitudinal studies from Sweden and the UK.[4 10] The Swedish data showed significant associations between the dust concentrations at onset of disease and the risk for asthma or rhinitis. The risk of asthma was increased at mean dust concentrations of 3 mg/m³, whereas the risk for rhinitis was increased at mean dust concentrations of 1 mg/m³, indicating an increased risk in all bakery work tasks. The British study is of case referent design, with the lowest exposed bakers (mean dust concentration 0.8 mg/m³) as reference. The bakers in the second exposure category (mean dust concentration 1.2 mg/m³) had increased—but not significantly so—risks for work related chest or eyes/nose symptoms, or sensitisation to flour or α amylase. The confidence intervals were of considerable width. In the highest exposure category (mean dust concentration 4.4 mg/m³) there were significantly increased risks for all outcomes (chest or eyes/nose symptoms, or sensitisation). Analyses with flour allergen exposure instead of inhalable dust gave very similar results.

WHAT DO WE WANT TO PREVENT?

Primarily we want to prevent baker's asthma. The definition of baker's asthma might have been fairly simple to the clinician some years ago: a baker with a history suggesting asthma and sensitisation by allergy tests to one or several cereal flours. However, developments in epidemiology and allergy have made this definition of baker's asthma questionable in the context of prevention as well as in clinical practice. Surveys of bakers have shown work related respiratory symptoms to occur without a demonstrable sensitisation to flour or α amylase. Cullinan *et al* did not find any sensitisation among nine bakers with lower respiratory tract symptoms of new onset.[11] Houba *et al* reported only 30% of bakers with work related symptoms to be sensitised.[12] This might be explained by sensitisation to other, as yet unknown bakery allergens, but another explanation is non-specific mucosal irritation caused by dust. The "latent allergy" phenomenon, where bakers become sensitised but without respiratory symptoms, also blurs the picture. There is little information in the literature on latent allergy, but it is indicated in the older studies where positive skin tests sometimes disappeared on follow up. On the other hand, sensitisation to flour or α amylase was a significant predictor (odds ratio 4.3) for work related symptoms in a longitudinal Italian study.[13] The severity and duration of baker's asthma may very well differ according to whether or not there is demonstrable sensitisation to bakery allergens, but this does not seem to have been studied.

Furthermore, one could argue it would be beneficial to prevent baker's rhinitis since it often precedes asthma, although the predictive value is not reported in the literature. Rhinitis itself also impairs quality of life.

PREVENTION BY MEDICAL SURVEILLANCE

The goal of medical surveillance is secondary prevention by early detection of a disease process before the occurrence of clinically adverse health outcomes. Although sometimes recommended, there is little scientific evidence of the effectiveness of medical surveillance programmes targeting occupational asthma. There is one study by Gordon *et al* which indicated that a questionnaire for detecting baker's asthma was not sensitive enough and detected only half the number of cases. Other possible tools for surveillance are lung function and allergy tests. Spirometry is an insensitive test for asthma. Serial monitoring of peak expiratory flow rate, repeated tests of non-specific bronchial reactivity or allergy tests are perhaps more sensitive surveillance methods, but are labour intensive when applied in bakery work forces. In the absence of scientific data supporting its preventive potential, it seems questionable to allocate resources to costly medical surveillance if it would endanger implementing primary prevention by reducing exposure.

PREVENTION BY MAXIMAL EXPOSURE LIMITS

Reduction of exposure to factors associated with a disease does not necessarily mean reduction of the risk for the disease.

Interventional studies are needed to test such hypotheses. However, exposure–response relations have been regarded as sufficient circumstantial support for reducing occupational exposures. Traditionally, bakery dust was often regarded as general or organic nuisance dust with standards set at concentrations of 5–10 mg/m^3. The reported exposure–response relations have initiated risk assessments in several countries: in the USA the American Conference of Governmental Industrial Hygienists adopted a threshold limit value (TLV) of 0.5 mg/m^3, the Dutch expert committee has proposed a limit of 0.5 mg/m^3, since January 2001 Sweden has set a limit of 3.0 mg/m^3, and Germany is presently revising its 4.0 mg/m^3 MAK (Maximale Arbeitsplatzkoncentration) value. These maximal exposure limits (MELs) are eight hour time weighted averages of inhalable dust by personal sampling.

The risk assessment for an MEL for dust is somewhat restricted by the grouping strategies and analytical methods used in the studies. For example, some studies compare "high" exposed groups with "low" exposed, but not with non-exposed. Heederik and Houba applied generalised additive modelling and smoothed plots of individual data in order to look for a possible exposure–response threshold for sensitisation to wheat.[14] They concluded that there was no threshold and that sensitisation could occur at flour dust concentrations of 0.5–1.0 mg/m^3.

Published exposure–response studies do not allow the identification of a NOAEL (no observable adverse effect level) for flour dust. Exposure to 3–6 mg/m^3 of inhalable dust increases the risk for respiratory tract symptoms (including asthma and rhinitis) and sensitisation to flour several fold. The risk estimates for symptoms at exposures < 3 mg/m^3 are sparse and of low precision because of the restrictions mentioned above. However, studies that have examined the effects of exposures < 3 mg/m^3 indicate increased risks—for example, the Dutch studies showed sensitisation at low levels, and the Swedish study demonstrated increased risk for rhinitis at 1 mg/m^3. Together with some safety margin, this indicates that an MEL should be in the range 0.5–1.0 mg/m^3 in order to prevent a substantial fraction of asthma, rhinitis, and sensitisation to flour.

So far MELs are expressed as dust. Dust in bakeries normally consists of flour to about 90%. Certain operations during confectionary work may emit dust with a high content of sugar, but this is usually evident from the task being performed. Consideration might be given to introducing an MEL based on the air concentration of flour allergen as a complement or instead of flour dust. The main argument against this approach is that most respiratory symptoms in bakers seem not to be related to flour sensitisation. Further obstacles are the diversity of allergens present in flour, without any single major allergen being identified, and the need for standardisation of the analyses. An MEL for fungal α amylase can also be considered since a standard for flour dust does not necessarily protect against sensitisation to α amylase. This has been highlighted by some measurements of high air concentrations of α amylase while dust levels were low. The risk assessment and management for α amylase may be different from that for flour. Progress in standardisation of the analytical process of α amylase was recently reported.[15]

DUST CONTROL

The key elements for dust control in bakeries are adequate local exhaust ventilation and good work practice. General dilution ventilation has only marginal effect on dust levels.

Baker's asthma: key points

Clinical

- Baker's asthma is often preceded by rhinitis, and skin symptoms are often concomitant
- Frequently there is atopy and sensitisation to flour and/or enzyme (for example, α amylase)
- Mechanisms behind cases without overt allergy to bakery allergens are unknown
- Risk is increased by high exposure to bakery dust

Management

- Reduce exposure by dust control or relocation
- Change of job to non-bakery work is often necessary
- Long term use of respirators is usually not feasible in bakeries

Prevention

- There is an exposure–response relation, meaning increased risks for baker's asthma, rhinitis, and sensitisation by exposure to flour or enzyme
- Today's MELs for flour dust (=3 mg/m^3) probably do not protect against baker's asthma
- Dust control in bakeries includes adequate local exhaust ventilation and good work practice. General dilution ventilation has only marginal effect on dust levels.

Local ventilation should be concentrated to flour release points such as weighing stations, dough making machines, dough brakes, and bread machines. Such ventilation can most probably reduce dust exposure to concentrations below 1 mg/m^3.[16] Work practice to avoid flour dust becoming airborne includes careful bag emptying and empty bag handling, and vacuum cleaning instead of using pressurised air. The introduction of new work practice requires that bakers are given training. An example is a training programme implemented in Switzerland. In order to minimise the need for throwing flour to prevent the dough sticking to work surfaces, technical changes such as using divider oils and flow tables must also can be considered.

MANAGEMENT

As in other forms of allergic asthma, the management of choice for the classic type of baker's asthma with sensitisation is allergen avoidance. This can be achieved by technical dust control, relocation of the baker to a less exposed job task, or by having the baker wear respiratory protection. Because of the abundance of dust in most bakeries in relation to the minute allergen exposure needed to elicit symptoms in sensitised workers, change of employment is often necessary. Symptomatic bakers without sensitisation can be helped by relocation to less exposed tasks if symptoms are caused by non-specific irritation. Respirators are in my experience seldom well tolerated by bakers because of the heat in bakeries and the hindering of physical activity. They also cause discomfort when worn for long periods.

Immunotherapy with flour has been reported to be successful in baker's asthma but needs further evaluation.

Management includes bakers seeking medical care because of symptoms but also the identification of at risk workers through surveys of bakers performed by occupational health services. In such surveys it is important to handle the outcome for the individual baker in a structured and, as far as possible, scientifically justified way. A Scandinavian workshop on the prevention of bakers' occupational diseases addressed these two aspects of management and the following recommendations were expressed.

- Asthmatics sensitised to flour or fungal α amylase should change to non-bakery employment
- Asthmatics without sensitisation to flour or fungal α amylase should be relocated to less exposed bakery tasks
- Bakers with rhinitis and sensitisation should be investigated closely and relocation to less exposed tasks should be considered
- Bakers sensitised to flour or fungal α amylase but without respiratory symptoms should be re-examined annually
- Bakers with rhinitis only but without sensitisation to bakery allergens do not warrant re-examination unless symptoms worsen.

ACKNOWLEDGEMENTS

I would like to thank Linnéa Lillienberg and Bengt Järvholm for comments on an earlier draft. The views expressed are entirely my own.

REFERENCES

1 **Houba R**, Doekes G, Heederick DJJ. Occupational respiratory allergy in bakery workers: a review of the literature. *Am J Ind Med* 1998;**34**:529–46.
- **Good review of the literature up to 1998, especially the epidemiology.**

2 **Brisman J**, Järvholm B. Occurrence of self-reported asthma among Swedish bakers. *Scand J Work Environ Health* 1995;**21**:487–93.
3 **Brisman J**, Järvholm B. Bakery work, atopy and the incidence of self-reported hay fever and rhinitis. *Eur Respir J* 1999;**13**:502–7.
4 **Cullinan P**, Cook A, Nieuwenhuijsen MJ, *et al*. Allergen and dust exposure as determinants of work-related symptoms and sensitization in a cohort of flour-exposed workers; a case-control analysis. *Ann Occup Hyg* 2001;**45**:97–103.
5 **Heederik D**, Newman Taylor AJ. Occupational asthma in the baking industry. In: Bernstein IL, Chan-Yeung M, Malo J-L, Bernstein DI, eds. *Asthma in the workplace*. New York: Marcel Dekker 1999:377–97.
- **Up to date review, with especially valuable sections on immunology and dust control.**

6 **Blands J**, Diamant B, Kallós-Deffner L, *et al*. Flour allergy in bakers: identification of allergenic fractions in flour and comparison of diagnostic methods. *Int Arch Allergy Appl Immunol* 1976;**52**:392–406.
7 **Sutton R**, Skerritt JH, Baldo BA, *et al*. The diversity of allergens involved in baker's asthma. *Clin Allergy* 1984;**14**:93–107.
8 **Baur X**, Fruhmann G, Haug G, *et al*. Role of Aspergillus amylase in bakers's asthma [letter]. *Lancet* 1986;i:43.
9 **Houba R**, Heederik D, Kromhout H. Grouping strategies for exposure to inhalable dust, wheat allergens and α-amylase allergens in bakeries. *Ann Occup Hyg* 1997;**41**:287–96.
- **Presents the background and results of an exposure grouping technique in bakeries.**

10 **Brisman J**, Järvholm B, Lillienberg L. Exposure-response relations for self reported asthma and rhinitis in bakers. *Occup Environ Med* 2000;**57**:335–40.
- **Discussion on exposure–response relations for baker's asthma and rhinitis.**

11 **Cullinan P**, Lowson D, Nieuwenhuijsen MJ, *et al*. Workrelated symptoms, sensitisation, and estimated exposure in workers not previously exposed to flour. *Occup Environ Med* 1994;**51**:579–83.
12 **Houba R**, Heederik D, Doekes G. Wheat sensitization and work-related symptoms in the baking industry are preventable. An epidemiologic study. *Am J Respir Crit Care Med* 1998;**158**:1499–503.
13 **De Zotti R**, Bovenzi M. Prospective study of work related respiratory symptoms in trainee bakers. *Occup Environ Med* 2000;**57**:58–61.
14 **Heederik D**, Houba R. An explanatory quantitative risk assessment for high molecular weight sensitizers: wheat flour. *Ann Occup Hyg* 2001;**45**:175–85.
- **Introduces risk assessment by general additive models in individual data.**

15 **Lillienberg L**, Baur X, Doekes G, *et al*. Comparison of four methods to assess fungal α-amylase in flour dust. *Ann Occup Hyg* 2000;**44**:421–33.
16 **Heinonen K**, Kulmala I, Säämänen A. Local ventilation for powder handling – combination of local supply and exhaust air. *Am Ind Hyg Assoc J* 1996;**57**:356–64.
- **Laboratory measurements and simulations on how to reduce flour dust exposure.**

QUESTIONS (SEE ANSWERS ON P 426)

(1) Which of the following statements on the present knowledge on exposure–response relations for baker's asthma is true?

(a) exposure–response relations for baker's asthma has not been studied
(b) there are no consistent results
(c) the risk for baker's asthma increases only at exposures to flour dust > 10 mg/m^3
(d) the risk for baker's asthma starts to increase at exposures to flour dust > 3 mg/m^3
(e) The risk for baker's asthma increases at exposures to flour dust < 0.5 mg/m^3.

(2) What is the proposed management of a case of baker's asthma with allergy to flour?

(a) re-examination annually
(b) more close investigation
(c) no action
(d) job change to non-bakery work
(e) long term use of respiratory protection

(3) Which of the following statements on allergens associated with baker's asthma is false?

(a) enzymes are powerful allergens
(b) storage mite allergy is a common cause of baker's asthma
(c) allergens in cereal flours and grasses are closely related
(d) not all bakers with sensitisation to flour have baker's asthma
(e) atopy increases the risk for sensitisation to flour

(4) Important factors for successful dust control in a bakery are:

(a) general ventilation
(b) local exhaust ventilation
(c) cleaning by using vacuum cleaning instead of pressurised air
(d) implementing a training programme in good work practice
(e) alternate techniques (such as divider oils) instead of throwing flour to prevent dough sticking

Additional references appear on the *Occupational and Environmental Medicine* website [www.occenvmed.com]

APMIS 111: 789–96, 2003
Printed in Denmark . All rights reserved

APMIS
ISSN 0903-4641

Dendritic cells and fungi

EVA BUENTKE and ANNIKA SCHEYNIUS

Department of Medicine, Unit of Clinical Allergy Research, Karolinska Institutet, Stockholm, Sweden

Buentke E, Scheynius A. Dendritic cells and fungi. APMIS 2003;111:789–96.

Fungi comprise a group of microorganisms that in the past 20 years has become increasingly important as a cause of human disease. Few fungi are professional but instead opportunistic pathogens, and some fungi can even act as allergens. Dendritic antigen-presenting cells function as a link between innate and adaptive immunity and are therefore important in recognition of pathogens. Effective defense requires the host to discriminate between different pathogens to induce an appropriate response. Signaling from different groups of microbes can be mediated via the Toll-like receptors (TLRs), leading to activation of conserved host defense signaling pathways that control the expression of a variety of immune response genes. Different dendritic cells (DCs) express different patterns of recognition molecules, which indicate that they are more or less efficient when responding to certain pathogens. DCs have an important role in the induction of cell-mediated immune responses to fungi, and the studies reviewed here show that fungi, or possibly fungi-derived factors, provide a powerful activation stimulus to DCs, resulting in DC maturation with upregulation of co-stimulatory molecules and production of cytokine patterns leading to different T cell responses. The possibility of using ex vivo-generated DCs as therapeutic tools for restoring anti-fungal immunity is a challenge for the future.

Key words: Dendritic cell activation; *Malassezia*; yeast; atopic eczema/dermatitis syndrome.

Annika Scheynius, Department of Medicine, Unit of Clinical Allergy Research, Karolinska Hospital L2:04, SE-171 76 Stockholm, Sweden. e-mail: annika.scheynius@ks.se

DENDRITIC CELLS

Dendritic cells (DCs) are key players in the initiation of adaptive immunity. They consist of a family of different subpopulations, which originate from the bone marrow and develop to perform different functions. At least two different subsets, the Langerhans cells (LCs) and the dermal DCs, reside in the skin (1). In the periphery, immature DCs act as sentinels that take up antigen upon antigen entry. When activated, the DCs mature and migrate to regional lymph nodes to activate naïve antigen-specific T cells (2). DCs are crucial also as modulators for the outcome of the immune response. As a link between the innate and adaptive immune systems they transfer important information, about the invading pathogen and the innate response in the periphery, to the T cells to evoke an appropriate response (3). A Th1- or a Th2-like response can be initiated by the DCs, or activation of regulatory T cells or Th3 cells can occur (4, 5). Different microorganisms will give rise to at least partly different immune responses (3). This can be detected already at the DC level, by studying how the different DCs respond to pathogens and antigens. The activation and maturation process involves lost ability to take up antigens, increased ability to present antigen-derived peptides, increased expression of co-stimulatory molecules, and production of cytokines (2).

Effective defense requires the host to discriminate between different pathogens to induce an appropriate response. Signaling from different groups of microbes can be mediated via the Toll-like receptors (TLRs). TLRs are signaling receptors with a critical role in sensing invading

microorganisms and recognizing pathogen-associated molecular patterns (6). The specificity of innate immune recognition in Drosophila is mediated by the Toll family of receptors, where Toll mediates anti-fungal responses (7). It has been shown that TLR2 is recruited specifically to macrophage phagosomes containing yeast, and that a point mutation in the receptor abrogates the inflammatory responses to yeast (8). Secretion of cytokines has also been reported to be dependent on TLR2 signaling (8, 9). Interestingly, myeloid DCs have been shown to express TLR2 (10, 11). It is therefore tempting to speculate that many of the responses to fungi seen in human DCs are mediated by TLR signaling. This, however, remains to be demonstrated. Different DCs express different patterns of TLRs, which indicates that they are more or less efficient when responding to certain pathogens (11). By studying DC activation, more can be learnt about the induction and tuning of immune responses, as well as about their role in induction of peripheral tolerance (12). We will here review recent data on how DCs interact with fungi.

FUNGI

Fungi are one of the three major classes of eukaryotic organisms. They comprise a group of microorganisms that in the past 20 years has become increasingly important as a cause of human disease (13). Ironically this is largely because of today's higher survival of immunocompromised patients. Thus, few fungi are professional pathogens, but are instead opportunistic pathogens (13).

Most fungi grow as thread-like hyphae, but this distinction is not definite as some fungi alternate between a yeast phase and a hyphal phase, depending on environmental stimuli (14). These fungi are termed dimorphic, meaning 'with two shapes', and exist in one form in nature and another form when causing infection (14). Many fungi pathogenic for man exhibit dimorphism (13). Yeasts are fungi that grow as single cells, producing daughter cells by budding or binary fission. Fungi commonly cause infection in immunosuppressed hosts, such as AIDS patients or patients who have received bone marrow transplants (15). Examples of yeasts and dimorphic fungi are: *Malassezia* species, *Candida albicans*, *Saccharomyces cerevisiae*, *Cryptococcus neoformans* and *Aspergillus fumigatus.*

Malassezia

The yeast *Malassezia*, formerly known as *Pityrosporum orbiculare/ovale*, belongs to the normal human skin flora (16, 17). It colonizes stratum corneum and is most abundant at sebum containing skin sites (18). Eight *Malassezia* species have been described so far, all present on human skin (19, 20). Although usually harmless, *Malassezia* species can cause skin infections and even systemic infections (17). A number of studies have shown that *Malassezia* is the causative agent of pityriasis versicolor and pityrosporum folliculitis and plays an important role in the pathogenesis of seborrhoeic dermatitis and atopic eczema/dermatitis syndrome (AEDS) (17, 19). AEDS is a chronic inflammatory skin disease that in its first phase is Th2 mediated, but in the chronic phase becomes Th1 mediated (21). Most healthy individuals have come into contact with *Malassezia* and mounted an IgG response to the yeast. IgE- and/or T cell reactivity to *Malassezia* is found in approximately 30–80% of AEDS patients, but rarely in patients with atopic respiratory diseases without AEDS (19). Nine *Malassezia* allergens have been identified and cloned so far, of which four show no sequence similarity to known proteins (19). The six *Malassezia* allergens cloned by us are now produced by recombinant techniques and used in diagnostic tests (22, 23). We recently demonstrated that a positive atopy patch test reaction in AEDS patients to *Malassezia* correlates with a Th2-like peripheral blood mononuclear cell response (22). The role of DCs in skewing these T cells has been partly addressed.

The skin barrier of AEDS patients is often disrupted (24), and whole *Malassezia* yeast cells and allergenic components from the yeast might pass the skin barrier and come into contact with professional antigen-presenting cells, such as the LCs. In order to prevent or improve conditions such as AEDS, studies of the initial steps in the interaction between DCs and *Malassezia* have been performed to gain knowledge about the events turning a non-sensitized individual into a sensitized one. Using monocyte-derived

dendritic cells (MDDCs) (25, 26) this interaction was studied in the human system. We have shown that the majority of immature MDDCs, reflecting LCs in the skin, internalized whole *Malassezia* yeast cells within 1 h (Fig. 1), whereas mature MDDCs, mimicking dendritic cells in the regional lymph nodes, had essentially lost the capacity to take up *Malassezia* and its components (27, 28). DCs express a number of receptors, such as the macrophage mannose receptor (MR) and Fc-receptors, which are involved in the interaction and uptake of microorganisms (29, 30). Uptake of *Malassezia* extract and mannan, an IgE-binding cell wall component that is highly immunogenic (31), was mainly mediated via the MR. Since yeast cell-wall components are highly mannosylated (32) this finding was not entirely unexpected. However, there were indications of an involvement of additional receptors (27). Uptake of the non-glycosylated recombinant allergen Mal s 5 (18 kDa), on the other hand, was mainly mediated via non-specific fluid phase pinocytosis (27). The process of internalizing *Malassezia* was associated with MDDC maturation, production of pro-inflammatory and immunoregulatory cytokines such as TNF-α, IL-1β and IL-18, but not IL-12p70, thus presumably favoring induction of a Th2-like immune response (28). IL-18 has generally been considered as a Th1-inducing cytokine and in mice been shown to synergize with IL-12 in inhibiting IgE production (33). In the absence of IL-12, however, it was reported that IL-18 stimulated IL-4 production and histamine release by mast cells, as well as IgE production (34–36). MDDCs incubated with *Malassezia* were also shown to induce proliferation in autologous lymphocytes, indicating that the MDDCs became functionally mature (28). This chain of events most likely contributes to the inflammatory reaction in AEDS. Furthermore, as these experiments were performed in the absence of IgE, they imply that sensitization of AEDS patients to *Malassezia* can be mediated by immature DCs in the skin.

To investigate whether DCs from AEDS patients already sensitized to *Malassezia* and healthy non-atopic individuals handle *Malassezia* differently upon encounter, we are currently performing a gene expression study. Preliminary results show that the DCs from the patients and the non-atopic individuals have a different gene expression pattern after stimulation with *Malassezia* yeast cells, where mRNA coding for several molecules involved in cell-cell adhesion, costimulation or with chemo-attracting properties is more highly expressed in the AEDS patients (37). We hypothesize that the skewing of the immune response to *Malassezia* towards a Th2-

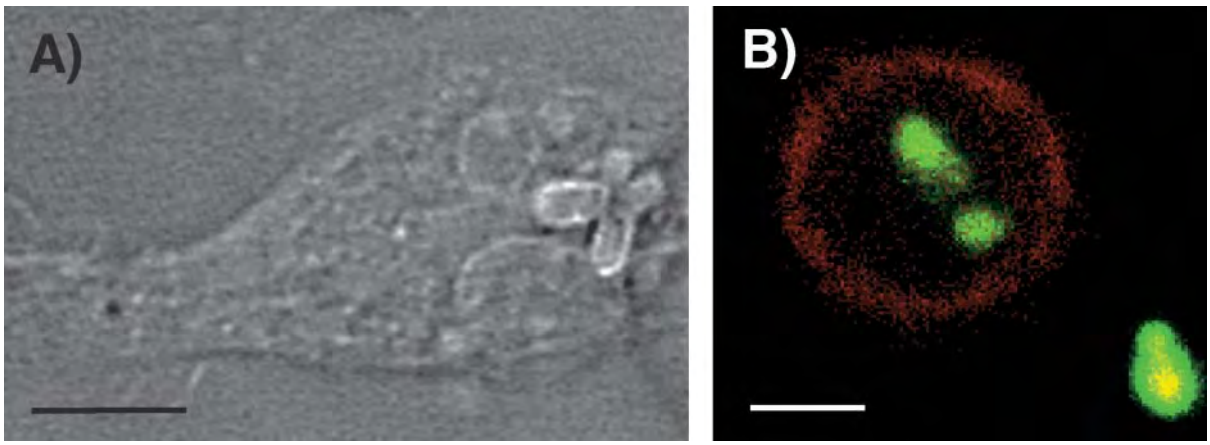


Fig. 1. Human immature MDDCs internalize whole *Malassezia* yeast cells. Time-lapse photography was used to study the kinetics of uptake of the yeast *Malassezia* by MDDCs (A). 2×10^5 immature MDDCs/ml were co-incubated with *Malassezia* yeast cells at a 1:5 ratio. Uptake usually occurred within 1 h. Internalization of yeast cells by immature $CD1a^+$ MDDCs was confirmed by confocal laser scanning microscopy (B). The image shows one optical section approximately through the middle of the MDDCs. Red is phycoerythrin conjugated mAb to CD1a and green is FITC-labeled yeast cells. Cross-talk between the detection channels has been compensated for using ImageSpace software. The scale bar in the lower left corner=10 μm.

like response is initiated already at the DC level by how the DCs respond to the yeast stimuli.

The microenvironment where the DCs encounter an antigen is important for the outcome of the immune response. Cells in the immune system communicate in a complex manner, and little is so far known about the possible modulation of DC function by other cells. Interestingly, reports have suggested a role for NK cells in affecting DC maturation and function upon direct contact between the cells (38–41). It was until recently not known if this interaction also takes place in vivo, or if a potential interaction of NK cells and DCs would be affected by allergen exposure of the DCs. We therefore studied the distribution of NK cells in the skin from AEDS patients sensitized to *Malassezia* with the emphasis on possible NK cell-DC interaction, and to assess whether stimulation of DCs with *Malassezia* would affect subsequent interaction between DCs and autologous NK cells. Using immunofluorescent techniques and confocal laser scanning microscopy, we could, for the first time, show close contact between NK cells ($CD56^+/CD3^-$) and $CD1a^+$ DCs in vivo, in biopsies from *Malassezia* atopy patch test-positive skin (42). DCs prestimulated with *Malassezia* yeast cells were shown to be less susceptible to NK cell-induced cell death, suggesting a direct effect imposed by *Malassezia* upon interaction of DCs with NK cells. Soluble factors from the yeast also influenced the NK-DC cross-talk by making NK cells less cytotoxic (42). These findings indicate that NK cells and DCs can interact in the skin, and that *Malassezia* seems to affect NK-DC interaction, directly through soluble factors acting on the NK cells and indirectly by maturing DCs. Our results show that the yeast *Malassezia* activates human DCs and makes them functionally mature, suggesting that DCs are important in host responses to *Malassezia.*

Candida

Candida albicans is a dimorphic fungus that inhabits mucosal membranes. Although part of our normal microflora, *Candida* can cause disease, and it is the most frequently isolated fungal pathogen in humans (13). Usually it grows as a yeast causing little or no damage, but under some circumstances this harmless commensal can become pathogenic, invading the mucosa and causing significant damage (13). This usually happens when a variety of predisposing factors renders the yeast able to escape the normal competition from resident bacteria and allows the yeast to multiply. It is commonly seen in AIDS patients and individuals who have had a prolonged course of antibacterial therapy. A more serious condition is systemic candidosis, when yeast cells proliferate in the circulatory system. This can, for example, occur after invasive surgical techniques.

A Th1-like response and subsequent cell-mediated immunity seems to mediate clearance of infection, whereas Th2-like responses are mainly associated with pathology (43). The decision making of the type of Th cell response initiated again lies on the DC. It has been shown that immature MDDCs can phagocytose *Candida*, and that the yeast is recognized via the MR (44). In 1 h, more than half of the MDDCs had ingested at least one *Candida* yeast cell, but the average was more than five yeast cells per MDDC. Opsonization did not enhance the uptake, and it was also shown that MDDCs kill the yeast regardless of whether it was opsonized or not (44). Internalized yeast cells were processed and MDDCs, as well as LCs and peritoneal DCs, have been shown to induce *Candida*-specific T cell proliferation (44–49). *Candida* can reside in two forms, as unicellular yeast and in a filamentous form as hyphae. In mice it has been demonstrated that the form of the microorganism clearly affects the DCs and the immune response. Both forms could be rapidly taken up by DCs, but whereas whole yeast cells induced IL-12 production and priming of Th1 cells, hyphae inhibited IL-12 and Th1 priming and induced IL-4 production from the DCs (50). Also in vivo protective anti-fungal immunity was induced only by DCs pulsed ex vivo with yeast cells but not with hyphae (50). In a model of human inflamed skin, *Candida* infection caused accumulation of $CD1a^+$ LCs in the dermis and in the draining lymph vessels, and consequently the number of $CD1a^+$ LCs in the epidermis decreased by half (51). The LCs in the dermis had a mature phenotype as demonstrated by CD83 and CD86 expression, and they were seen in close contact with memory T cells (51). These studies suggest that DCs play an important role in host response to *Candida* infection, and that different forms of the yeast differentially activate them.

The use of microarrays to measure gene expression profiles of DCs in response to different pathogens has opened up a new way to explore how DCs modulate the immune system in response to different pathogens. The response to *Escherichia coli*, *C. albicans*, and influenza virus and their molecular components was compared in one study (52). Both a shared response and a pathogen-specific gene expression were observed upon exposure to the pathogens. Interestingly, there were no genes that were specifically induced only by the yeast. This study further reveals that human DCs distinguish between pathogens and elicit tailored pathogen-specific immune responses (52).

Saccharomyces cerevisiae

Saccharomyces cerevisiae, commonly known as bakers' yeast, rarely causes disease in humans. Immature human DCs, mice LCs, and bone marrow-derived DCs from mice have been reported to take up the yeast *S. cerevisiae* (53–55). The yeast cells were internalized within 2–4 h and appeared largely degraded, as detected by decrease in specific fluorescence intensity from the yeast, after 24 h (54, 55). The majority of the human DCs had ingested the yeast, and around 40% of them had internalized more than 15 yeast (55). The interaction was shown to be mediated by the MR and lead to DC maturation and IL-12p70 production (53–55). The expression of co-stimulatory molecules such as CD80 and CD86, as well as expression of CD40, CD54 and MHC class-II, increased several fold after stimulation with yeast for 48 h (54). The interaction led to efficient priming of MHC class-I- and class-II-restricted, antigen-specific T cell responses (54). Human MDDCs have also been shown to take up yeast oligomannosides very efficiently, once again highlighting the importance of the MR in DC-yeast interactions (56). The fact that yeast can easily be engineered to express foreign antigens, together with the potency of the yeast to induce DC maturation, makes it a promising tool in generating DC-based vaccines for stimulation of cell-mediated immunity (54).

Cryptococcus neoformans

Cryptococcus neoformans is a significant pathogen in immunocompromised patients, causing the disease termed cryptococcosis (57). This disease occurs in about 7–8% of AIDS patients in the USA, and a slightly smaller percentage (3–6%) in Western Europe. The capsule is a significant virulence determinant of *C. neoformans* because it helps to prevent the cells from being recognized and engulfed by white blood cells. It infects through the lungs, where it causes a mild or chronic, persistent pneumonia, depending on the person's degree of immunity. In a recent publication, human DCs were shown to take up *C. neoformans* via the MR and FcγRII for presentation to T cells (58). The role of different DC subsets in the response to the yeast was investigated in a mouse study (59). The three subsets studied were LCs, myeloid DCs, and lymphoid DCs. The aim was to investigate if different DC subsets were associated with the development of protective versus non-protective cell-mediated immune responses against the fungal pathogen. Mice were immunized with protective (cryptococcal culture filtrate Ag-CFA) and/or non-protective immunogens (heat-killed cryptococci-CFA). The protective immunogen, in contrast to the non-protective immunogen or controls, stimulated significant increases in LCs, myeloid DCs, lymphoid DCs and activated $CD4^+$ T cells in draining lymph nodes (59). The results indicated that lymph node LCs and myeloid DCs were needed for induction of the protective response, and that the ratio of LCs:myeloid DCs in draining lymph nodes was modulated by the cryptococcal antigen and determined whether protective or non-protective anti-cryptococcal cell-mediated responses developed (59).

Aspergillus fumigatus

Aspergillus fumigatus is a fungus that can cause pulmonary infections upon inhalation of conidia. In immunocompromised patients, *A. fumigatus* infection can lead to invasive pulmonary aspergillosis. A Th1/Th2 dysregulation, with a switch to a Th2-like immune response, may contribute to the development and unfavorable outcome of this disease. The fungi contain allergens and have been shown to mediate allergic bronchopulmonary aspergillosis, a life-threatening hypersensitivity disease (60). Mouse DCs have been demonstrated to internalize conidia and hyphae from *A. fumigatus* (61). This was mediated through

distinct phagocytic mechanisms and recognition receptors. Conidia were internalized through the MR and possibly DEC-205, whereas hyphae were taken up through the complement receptor 3 and FcγR. The DCs could also discriminate between the different forms of the yeast as detected by cytokine production. IL12p70 was produced when the DCs had been stimulated with conidia, and IL-4 and IL-10 when stimulated with hyphae. Both forms, however, induced TNFα production. DCs underwent functional maturation in vivo upon migration to the draining lymph nodes and spleens (61). The DCs instructed local and peripheral Th cell reactivity to the fungus. The conidia induced Th1-like, and the hyphae induced Th2-like responses (61). Surface expression of HLA-DR, CD80 and CD86 increased on human MDDCs after 24 h interaction with *Aspergillus* conidia, and IL-12 production was induced (62). DCs pretreated with conidia stimulated lymphocyte proliferation and production of high levels of IFNγ, but not interleukin-10, implying that DCs evoke a Th1-like response to be mounted against *Aspergillus* (62). DCs generated from $CD34^+$ progenitors collected prior to stem cell transplantation could partially restore the in vitro anti-fungal proliferative response of lymphocytes obtained from patients after transplantation (62).

CONCLUDING REMARKS

DCs have an important role in the induction of cell-mediated immune responses to fungi. The studies reviewed here show that fungi, or possibly fungi-derived factors, provide a powerful activation stimulus to DCs, resulting in DC maturation with upregulation of co-stimulatory molecules and production of cytokines leading to different T cell responses. The ability of DCs to induce different immune responses depending on the form of the fungi might be better understood after further investigations of the role of TLR signaling. Studies also suggest that ex vivo-generated DC might be useful in restoring or enhancing the anti-fungal immunity, highlighting the fast growing area of research on the use of DCs as therapeutic tools in the future.

This work was supported by grants from the Swedish Medical Research Council (project no 16x-7924), the Swedish Council for Work Life Sciences, the Swedish Asthma and Allergy Association's Research Foundation, the Swedish Foundation for Health Care Sciences and Allergy Research, the Queen Silvia Jubilee Foundation, the Hesselman Foundation, and Karolinska Institutet.

REFERENCES

1. Sallusto F. Origin and migratory properties of dendritic cells in the skin. Curr Opin Allergy Clin Immunol 2001;1:441–8.
2. Banchereau J, Briere F, Caux C, Davoust J, Lebecque S, Liu YJ, et al. Immunobiology of dendritic cells. Annu Rev Immunol 2000;18:767–811.
3. Pulendran B, Palucka K, Banchereau J. Sensing pathogens and tuning immune responses. Science 2001;293:253–6.
4. de Jong EC, Vieira PL, Kalinski P, Schuitemaker JH, Tanaka Y, Wierenga EA, et al. Microbial compounds selectively induce Th1 cell-promoting or Th2 cell-promoting dendritic cells in vitro with diverse th cell-polarizing signals. J Immunol 2002; 168:1704–9.
5. Weiner HL. Induction and mechanism of action of transforming growth factor-beta- secreting Th3 regulatory cells. Immunol Rev 2001;182: 207–14.
6. Medzhitov R, Janeway C Jr. Innate immune recognition: mechanisms and pathways. Immunol Rev 2000;173:89–97.
7. Hoffmann JA, Reichhart JM. Drosophila innate immunity: an evolutionary perspective. Nat Immunol 2002;3:121–6.
8. Underhill DM, Ozinsky A, Hajjar AM, Stevens A, Wilson CB, Bassetti M, et al. The Toll-like receptor 2 is recruited to macrophage phagosomes and discriminates between pathogens. Nature 1999;401:811–5.
9. Prebeck S, Kirschning C, Durr S, da Costa C, Donath B, Brand K, et al. Predominant role of toll-like receptor 2 versus 4 in Chlamydia pneumoniae-induced activation of dendritic cells. J Immunol 2001;167:3316–23.
10. Muzio M, Bosisio D, Polentarutti N, D'Amico G, Stoppacciaro A, Mancinelli R, et al. Differential expression and regulation of toll-like receptors (TLR) in human leukocytes: selective expression of TLR3 in dendritic cells. J Immunol 2000; 164:5998–6004.
11. Kadowaki N, Ho S, Antonenko S, Malefyt RW, Kastelein RA, Bazan F, et al. Subsets of human dendritic cell precursors express different toll-like receptors and respond to different microbial antigens. J Exp Med 2001;194:863–9.

12. Albert ML, Jegathesan M, Darnell RB. Dendritic cell maturation is required for the cross-tolerization of CD8+ T cells. Nat Immunol 2001;2:1010–7.
13. van Burik JA, Magee PT. Aspects of fungal pathogenesis in humans. Annu Rev Microbiol 2001;55:743–72.
14. Wendland J. Comparison of morphogenetic networks of filamentous fungi and yeast. Fungal Genet Biol 2001;34:63–82.
15. Jahagirdar BN, Morrison VA. Emerging fungal pathogens in patients with hematologic malignancies and marrow/stem-cell transplant recipients. Semin Respir Infect 2002;17:113–20.
16. Midgley G. The diversity of *Pityrosporum* (*Malassezia*) yeasts in vivo and in vitro. Mycopathologia 1989; 106:143–53.
17. Faergemann J. *Pityrosporum* species as a cause of allergy and infection. Allergy 1999;54:413–9.
18. Faergemann J, Aly R, Maibach HI. Quantitative variations in distribution of *Pityrosporum orbiculare* on clinically normal skin. Acta Derm Venereol 1983;63:346–8.
19. Scheynius A, Johansson C, Buentke E, Zargari A, Linder MT. Atopic eczema/dermatitis syndrome and *Malassezia.* Int Arch Allergy Immunol 2002;127:161–9.
20. Sugita T, Takashima M, Shinoda T, Suto H, Unno T, Tsuboi R, et al. New yeast species, *Malassezia dermatis*, isolated from patients with atopic dermatitis. J Clin Microbiol 2002;40: 1363–7.
21. Ring J, Darsow U, Behrendt H. Atopic eczema and allergy. Curr Allergy Rep 2001;1:39–43.
22. Johansson C, Eshaghi H, Linder MT, Jakobson E, Scheynius A. Positive atopy patch test reaction to Malassezia furfur in atopic dermatitis correlates with a T helper 2-like peripheral blood mononuclear cells response. J Invest Dermatol 2002;118:1044–51.
23. Zargari A, Eshaghi H, Bäck O, Johansson S, Scheynius A. Serum IgE reactivity to *Malassezia furfur* extract and recombinant *M. furfur* allergens in patients with atopic dermatitis. Acta Derm Venereol 2001;81:418–22.
24. Rajka G. Essential Aspects of Atopic Dermatitis. Springer-Verlag, Berlin Heidelberg, 1989.
25. Romani N, Gruner S, Brang D, Kampgen E, Lenz A, Trockenbacher B, et al. Proliferating dendritic cell progenitors in human blood. J Exp Med 1994;180:83–93.
26. Sallusto F, Lanzavecchia A. Efficient presentation of soluble antigen by cultured human dendritic cells is maintained by granulocyte/macrophage colony-stimulating factor plus interleukin 4 and downregulated by tumor necrosis factor alpha. J Exp Med 1994;179:1109–18.
27. Buentke E, Zargari A, Heffler LC, Avila-Carino J, Savolainen J, Scheynius A. Uptake of the yeast *Malassezia furfur* and its allergenic components by human immature CD1a+ dendritic cells. Clin Exp Allergy 2000;30:1759–70.
28. Buentke E, Heffler LC, Wallin RP, Löfman C, Ljunggren HG, Scheynius A. The allergenic yeast *Malassezia furfur* induces maturation of human dendritic cells. Clin Exp Allergy 2001;31:1583–93.
29. Clark GJ, Angel N, Kato M, Lopez JA, MacDonald K, Vuckovic S, et al. The role of dendritic cells in the innate immune system. Microbes Infect 2000;2:257–72.
30. Sallusto F, Cella M, Danieli C, Lanzavecchia A. Dendritic cells use macropinocytosis and the mannose receptor to concentrate macromolecules in the major histocompatibility complex class II compartment: downregulation by cytokines and bacterial products. J Exp Med 1995; 182:389–400.
31. Doekes G, Kaal MJ, van Ieperen-van Dijk AG. Allergens of *Pityrosporum ovale* and *Candida albicans.* II. Physicochemical characterization. Allergy 1993;48:401–8.
32. Lintu P, Savolainen J, Kalimo K. IgE antibodies to protein and mannan antigens of *Pityrosporum ovale* in atopic dermatitis patients. Clin Exp Allergy 1997;27:87–95.
33. Yoshimoto T, Okamura H, Tagawa YI, Iwakura Y, Nakanishi K. Interleukin 18 together with interleukin 12 inhibits IgE production by induction of interferon-gamma production from activated B cells. Proc Natl Acad Sci USA 1997;94:3948–53.
34. Yoshimoto T, Tsutsui H, Tominaga K, Hoshino K, Okamura H, Akira S, et al. IL-18, although antiallergic when administered with IL-12, stimulates IL-4 and histamine release by basophils. Proc Natl Acad Sci USA 1999;96:13962–6.
35. Yoshimoto T, Mizutani H, Tsutsui H, Noben-Trauth N, Yamanaka K, Tanaka M, et al. IL-18 induction of IgE: dependence on $CD4^+$ T cells, IL-4 and STAT6. Nat Immunol 2000;2:132–7.
36. Nakanishi K, Yoshimoto T, Tsutsui H, Okamura H. Interleukin-18 regulates both Th1 and Th2 responses. Annu Rev Immunol 2001;19:423–74.
37. Gabrielsson S, Buentke E, Liedén A, Schmidt M, D'Amato M, Tengvall-Linder M, et al. Altered cDNA gene expression in *Malassezia sympodialis* stimulated dendritic cells in atopic eczema/dermatitis syndrome. Allergy 2002;57:7.
38. Ferlazzo G, Tsang ML, Moretta L, Melioli G, Steinman RM, Munz C. Human dendritic cells activate resting natural killer (NK) cells and are recognized via the NKp30 receptor by activated NK cells. J Exp Med 2002;195:343–51.
39. Gerosa F, Baldani-Guerra B, Nisii C, Marchesini V, Carra G, Trinchieri G. Reciprocal activating interaction between natural killer cells and dendritic cells. J Exp Med 2002;195:327–33.

40. Piccioli D, Sbrana S, Melandri E, Valiante NM. Contact-dependent stimulation and inhibition of dendritic cells by natural killer cells. J Exp Med 2002;195:335–41.
41. Wilson JL, Heffler LC, Charo J, Scheynius A, Bejarano MT, Ljunggren HG. Targeting of human dendritic cells by autologous NK cells. J Immunol 1999;163:6365–70.
42. Buentke E, Heffler LC, Wilson J, Wallin RPA, Löfman C, Chambers BJ, Ljunggren HG, Scheynius A. Natural killer and dendritic cell contact in lesional atopic dermatitis skin – *Malassezia*-influenced cell interaction. J Invest Dermatol 2002;119:850–7.
43. Puccetti P, Romani L, Bistoni F. A TH1-TH2-like switch in candidiasis: new perspectives for therapy. Trends Microbiol 1995;3:237–40.
44. Newman SL, Holly A. *Candida albicans* is phagocytosed, killed, and processed for antigen presentation by human dendritic cells. Infect Immun 2001;69:6813–22.
45. Chen B, Shi Y, Smith JD, Choi D, Geiger JD, Mule JJ. The role of tumor necrosis factor alpha in modulating the quantity of peripheral blood-derived, cytokine-driven human dendritic cells and its role in enhancing the quality of dendritic cell function in presenting soluble antigens to CD4+ T cells in vitro. Blood 1998;91:4652–61.
46. Goxe B, Latour N, Bartholeyns J, Romet-Lemonne JL, Chokri M. Monocyte-derived dendritic cells: development of a cellular processor for clinical applications. Res Immunol 1998;149: 643–6.
47. Cohen PJ, Katz SI. Cultured human Langerhans cells process and present intact protein antigens. J Invest Dermatol 1992;99:331–6.
48. Betjes MG, Tuk CW, Struijk DG, Krediet RT, Arisz L, Beelen RH. Antigen-presenting capacity of macrophages and dendritic cells in the peritoneal cavity of patients treated with peritoneal dialysis. Clin Exp Immunol 1993;94:377–84.
49. Bjercke S, Elgo J, Braathen L, Thorsby E. Enriched epidermal Langerhans cells are potent antigen-presenting cells for T cells. J Invest Dermatol 1984;83:286–9.
50. d'Ostiani CF, Del Sero G, Bacci A, Montagnoli C, Spreca A, Mencacci A, et al. Dendritic cells discriminate between yeasts and hyphae of the fungus *Candida albicans.* Implications for initiation of T helper cell immunity in vitro and in vivo. J Exp Med 2000;191:1661–74.
51. Katou F, Ohtani H, Saaristo A, Nagura H, Motegi K. Immunological activation of dermal Langerhans cells in contact with lymphocytes in a model of human inflamed skin. Am J Pathol 2000;156:519–27.
52. Huang Q, Liu D, Majewski P, Schulte LC, Korn JM, Young RA, et al. The plasticity of dendritic cell responses to pathogens and their components. Science 2001;294:870–5.
53. Reis e Sousa C, Stahl PD, Austyn JM. Phagocytosis of antigens by Langerhans cells in vitro. J Exp Med 1993;178:509–19.
54. Stubbs AC, Martin KS, Coeshott C, Skaates SV, Kuritzkes DR, Bellgrau D, et al. Whole recombinant yeast vaccine activates dendritic cells and elicits protective cell-mediated immunity. Nat Med 2001;7:625–9.
55. Boyer A, Andreu G, Romet-Lemonne JL, Fridman WH, Teillaud JL. Generation of phagocytic MAK and MAC-DC for therapeutic use: characterization and in vitro functional properties. Exp Hematol 1999;27:751–61.
56. Bedouet L, Bousser MT, Frison N, Boccaccio C, Abastado JP, Marceau P, et al. Uptake of dimannoside clusters and oligomannosides by human dendritic cells. Biosci Rep 2001;21:839–55.
57. Mitchell TG, Perfect JR. Cryptococcosis in the era of AIDS – 100 years after the discovery of Cryptococcus neoformans. Clin Microbiol Rev 1995;8:515–48.
58. Syme RM, Spurrell JC, Amankwah EK, Green FH, Mody CH. Primary dendritic cells phagocytose *Cryptococcus neoformans* via mannose receptors and Fcgamma receptor II for presentation to T lymphocytes. Infect Immun 2002;70: 5972–81.
59. Bauman SK, Nichols KL, Murphy JW. Dendritic cells in the induction of protective and nonprotective anticryptococcal cell-mediated immune responses. J Immunol 2000;165:158–67.
60. Crameri R, Blaser K. Allergy and immunity to fungal infections and colonization. Eur Respir J 2002;19:151–7.
61. Bozza S, Gaziano R, Spreca A, Bacci A, Montagnoli C, di Francesco P, et al. Dendritic cells transport conidia and hyphae of *Aspergillus fumigatus* from the airways to the draining lymph nodes and initiate disparate Th responses to the fungus. J Immunol 2002;168:1362–71.
62. Grazziutti M, Przepiorka D, Rex JH, Braunschweig I, Vadhan-Raj S, Savary CA. Dendritic cell-mediated stimulation of the in vitro lymphocyte response to *Aspergillus.* Bone Marrow Transplant 2001;27:647–52.

MAJOR ARTICLE

Invasive *Saccharomyces* Infection: A Comprehensive Review

Adela Enache-Angoulvant[1,2] and Christophe Hennequin[1,2]

[1]Laboratoire de Parasitologie, Faculté de Médecine Pierre et Marie Curie, Université Pierre et Marie Curie, and [2]Service de Parasitologie, Hôpital Tenon, Paris, France

Background. *Saccharomyces cerevisiae* (also known as "baker's yeast" or "brewer's yeast") is mostly considered to be an occasional digestive commensal. However, since the 1990s, there have been a growing number of reports about its implication as an etiologic agent of invasive infection. A particular feature of such infections is their association with a probiotic preparation of *Saccharomyces boulardii* (a subtype of *S. cerevisiae*) for treatment various diarrheal disorders.

Methods. We collected published case reports, through May 2005, of invasive *Saccharomyces* infection by use of a Medline query. Epidemiological and clinical charts and therapeutic strategies were analyzed.

Results. We found 92 cases of *Saccharomyces* invasive infection. Predisposing factors were similar to those of invasive candidiasis, with intravascular catheter and antibiotic therapy being the most frequent. Blood was the most frequent site of isolation (for 72 patients). *S. boulardii* accounted for 51.3% of fungemias and was exclusively isolated from blood. Compared with patients infected with *S. cerevisiae,* patients infected with *S. boulardii* were more frequently immunocompetent and had a better prognosis. *Saccharomyces* invasive infection was clinically indistinguishable from an invasive candidiasis. Overall, *S. cerevisiae* clinical isolates exhibited low susceptibility to amphotericin B and azole derivatives. However, global outcome was favorable in 62% of the cases. Treatment with intravenous amphotericin B and fluconazole, in combination with central vascular catheter removal, were effective therapeutic options.

Conclusion. *Saccharomyces* organisms should now be added to the growing list of emerging fungal pathogens. Special caution should be taken regarding the use of *S. boulardii* probiotic preparations.

Saccharomyces cerevisiae (also known as "baker's yeast" or "brewer's yeast") is widespread in nature and can be found on plants and fruit and in soil [1]. *S. cerevisiae* is now included in some diet or health foods. *Saccharomyces boulardii,* a subtype of *S. cerevisiae,* is also used in probiotic preparation for the prevention and treatment of various diarrheal disorders, such as those associated with *Clostridium difficile* infection or parenteral nutrition [2]. On the basis of results of molecular studies, *S. boulardii* is now considered to be an invalid taxon and must be considered as either a subtype [3] or a variety [4] of *S. cerevisiae.* It is not routinely possible to identify *S. boulardii,* because its phenotypic (auxotrophic) characteristics are not pathognomonic of the subtype. Although restriction-enzyme analysis of mitochondrial DNA [5, 6] or karyotyping [7, 8] are the tests most frequently used to identify *S. boulardii,* these methods have yet to be fully evaluated, notably for their discriminatory power and, thus, for their ability to unambiguously identify *S. boulardii.* In our experience, internal transcribed spacer sequencing has failed to discriminate *S. boulardii* from some *S. cerevisiae* strains [9] (C.H., unpublished data). To our knowledge, determination of the length of a particular microsatellite-containing locus, which was performed by Hennequin et al. [10] on 67 *S. cerevisiae* strains and 24 *S. boulardii* isolates, is the only published method that reliably distinguishes between *S. boulardii* and other *S. cerevisiae* strains.

The rate of *Saccharomyces* carriage varies according to the populations investigated. A study from the 1940s reported that the respiratory tract of 7% of patients with chronic pulmonary disease was colonized with *Saccharomyces* organisms [11]. Occasionally, *S. cerevisiae* can be isolated from the vaginal flora (0.9%–5.8%

Received 21 April 2005; accepted 20 July 2005; electronically published 1 November 2005.

Reprints or correspondence: Dr. Christophe Hennequin, Laboratoire de Parasitologie, Faculté de Médicine Pierre et Marie Curie, 75012 Paris, France (christophe.hennequin@laposte.net).

Clinical Infectious Diseases 2005; 41:1559–68

1058-4838/2005/4111-0013$15.00

Table 1. Clinical and microbiologic features of 54 patients with *Saccharomyces cerevisiae* invasive infections.

Infection type, patient, reference	Age, sex	Site of isolation or associated condition		Concomitantly isolated organisms	Underlying condition	Predisposing factor			Treatment	IVC removal	Outcome
		First isolate	Additional isolates			NeutP	IVC	ATB			
Disseminated											
1 [23]	54 years, F	Blood	Urine	Diphtheroids	Valvular prosthesis	No	Yes	Yes	AmB	Yes	Favorable
2 [24]	38 years, M	Blood	…	None	IVDA, valvular prosthesis	No	NS	Yes	AmB	NS, VR	Favorable
3 [25]	68 years, M	Bone marrow[c]	Urine	None	Ingestion of brewer's yeast	Yes	NS	No	None	NS	Favorable
4 [26]	59 years, M	Blood	Esophagus	None	Severe burns	Yes	Yes	Yes	AmB	Yes	Favorable
5 [27]	61 years, M	Blood	CVC	None	Renal failure, hemodialysis, abdominal surgery	No	Yes	Yes	MCZ, 5-FC	Yes	Death
6 [28]	37 years, F	Blood	Chorioretinitis	None	AIDS, IVDA, peritoneal dialysis	NS	NS	Yes	AmB	NS	Favorable
7 [29]	26 years, M	Blood	…	NS	AML	No	Yes	NS	NS	Yes	Favorable
8 [29]	81 years, F	Blood	…	NS	AML	Yes	NS	NS	AmB	NS	Favorable
9 [30]	25 years, F	Blood	…	None	Trauma, abdominal surgery	No	Yes	Yes	AmB	Yes	Favorable
10 [31]	39 years, M	Lung[c]	Spleen,[c] digestive tract	*Pneumocystis carinii, Mycobacterium avium-intracellulare*	AIDS	No	No	No	None	No	Death
11 [32]	6 weeks, NS	Blood	CVC, urine	*Candida albicans*	Abdominal surgery	No	Yes	Yes	None	Yes	Favorable
12 [32]	71 years, M	Blood	Throat	*Kluyveromyces marxianus, Geotrichum capitatum*	Aplastic anemia	Yes	Yes	Yes	AmB, 5-FC	NS	Death
13 [33]	62 years, F	Blood	Liver abscess, biliary fluid	*Candida parapsilosis* from liver and biliary fluid	Pancreas neoplasia, digestive surgery	NS	NS	Yes	AmB	NS	Death
14 [33]	65 years, F	Blood	Heart,[c] pericardium,[c] lung,[c] serosa of colon[c]	*Aspergillus* species from heart and pericardium	Hemopathy (idiopathic pancytopenia)	Yes	NS	Yes	None	NS	Death
15 [34]	71 years, M	Blood	…	None	Epidermoid cancer, chemotherapy	No	NS	Yes	AmB, 5-FC[a]	NS	Death
16 [35]	70 years, F	Blood	…	None	Hemopathy (EBRA)	Yes	NS	Yes	None	NS	Death
17 [22]	8 weeks, F	Blood	…	*Enterobacter cloacae*	Respiratory failure, ECMO	NS	NS	Yes	None	NS	Favorable
18 [36]	48 years, F	Blood	…	None	Allogenic BMT for CML	No	Yes	NS	FLU	Yes	Favorable
19 [37]	NS, NS	Endocarditis[c]	Blood	NS	Prosthetic valve	NS	Yes	Yes	AmB	NS	Favorable
20 [38]	32 years, F	Blood	…	None	Right breast abscess, septic shock	NS	NS	NS	NS	NS	Favorable
21 [38]	16 years, M	Blood	…	None	Convulsion seizure	NS	NS	NS	NS	NS	Favorable
22 [39]	NS, NS	Blood	…	NS	Chronic alcoholism, root canal treatment	NS	NS	Yes	NS	NS	Favorable
23 [40]	NS, NS	Blood	…	NS	AIDS	NS	NS	NS	NS	NS	Death
24 [41]	34 years, M	Blood	…	None	Relapsed ALL	Yes	NS	Yes	AmB, 5-FC[a]	NS	Favorable
25 [42]	NS, NS	Blood	…	NS	BMT	NS	Yes	NS	NS	NS	NS
26 [43]	10 years, F	Blood	Lung,[c] mitral valve[c]	None	Cystic fibrosis, intestinal obstruction, ileostomy	NS	Yes	Yes	AmB	Yes	Death
27 [43]	10 weeks, NS	Blood	…	*Klebsiella pneumoniae*	Premature birth, CRD, corticosteroid therapy, gastrostomy	No	Yes	Yes	AmB	Yes	Favorable
28 [43]	7 years, M	Blood	…	*K. pneumoniae, E. cloacae*	Gastroschisis, intestinal resection, T cell lymphopenia	Yes	NS	No	AmB	Yes	Favorable
29 [44]	85 years, M	Blood	…	None	Trauma	No	Yes	NS	AmB	NS	Favorable

30 [45]	59 years, F	Blood	Peritoneum, vagina, respiratory tract, liver abscess	*Enterococcus faecium*	Diabetes, chronic alcoholism, choledocojejunostomy	No	NS	Yes	FLU then AmB	NS	Death
31 [46]	2 years, F	Endocarditis[c]	Blood	None	Fallot tetralogy	No	NS	NS	None	NS	Death
32 [47]	8 months, M	Blood	…	NS	AML, cytotoxic chemotherapy	Yes	Yes	Yes	AmB[a]	Yes	Favorable
33 [48]	2 weeks, F	Blood	…	NS	Premature birth, gastrointestinal symptoms	No	NS	Yes	AmB	NS	Favorable
34 [49]	30 years, F	Blood	…	NS	Allogenic BMT for CML	NS	NS	NS	AmB	NS	Favorable
35 [50]	57 years, F	Blood	Endocarditis[c]	None	Prosthetic valve	No	No	Yes	KET	No, VR	Death
36 [51]	56 years, M	Blood	…	NS	Aortaenteric fistula	No	NS	Yes	AmB	NS	Death
37 [52]	9 days, M	Endocarditis[c]	Blood	None	Premature birth, ductus arteriosus, necrotizing enterocolitis	No	Yes	Yes	AmB, FLU	NS	Favorable
Localized											
38 [53]	73 years, F	Synovial fluid (arthritis)	…	*Staphylococcus epidermidis*	Rheumatoid arthritis, Sjögren syndrome	No	NS	No	None	Favorable	NS
39 [54]	66 years, M	Retrogastric abscess	…	None	Surgery for pancreatic neoplasia extrahepatic biliary obstruction	No	NS	Yes	KET	NS	NS
40 [20]	75 years, M	Periurethral fistula drainage[c]	…	*Proteus vulgaris, Enterococcus* species	Diabetes, chronic renal failure, prostatic neoplasia	No	NS	No	NS	NS	Favorable
41 [20]	52 years, M	Pleural fluid	…	α-Hemolytic streptococci, *Escherichia coli, Lactobacillus* species, *Staphylococcus aureus*	COPD, corticosteroid therapy, ingestion of yeast	NS	NS	No	NS	NS	Death
42 [20]	70 years, M	Renal abscess[c]	Urine	*C. albicans*	Diabetes, Foley catheter	NS	NS	No	KET	NS	NS
43 [55]	NS, NS	Lung[c]	…	NS	Hodgkin disease	NS	NS	NS	NS	NS	Death
44 [56]	17 years, M	Perihilar mass[c]	Bronchoscopic specimens, esophageal lesions	*M. avium-intracellulare*	AIDS	NS	NS	NS	AmB[a]	Death	Death
45 [33]	68 years, M	Lung[c]	…	Coagulase-negative staphylococci	AML, chemotherapy	Yes	NS	No	AmB then KET	NS	NS
46 [57]	60 years, F	Pleural fluid	Sputum	None	Cirrhosis, esophagopleural fistula	No	NS	No	AmB	NS	Death
47 [58]	33 years, M	Peritoneum[c]	…	None	CAPD	NS	Yes	No	FLU	Yes	Favorable
48 [49]	36 years, M	Lung[c]	…	None	Acute leukemia, allogenic BMT	NS	Yes	NS	NS	NS	Favorable
49 [22]	70 years, M	Abdominal abscess[c]	…	*Enterobacter aerogenes*	Abdominal surgery for abdominal aortic aneurysm, rhabdomyolysis	NS	NS	Yes	FLU (for 2 days) then AmB	NS	Favorable
50 [59]	67 years, M	Ureter[c] (fungus ball)	Urine, oral swab	NS	Surgery for urothelial carcinoma, prostatic adenoma	NS	NS	Yes	ITR, 5-FC, AmB irrigation	NS	Favorable
51 [60]	4 years, M	Mandibular osteomyelitis[c]	…	NS	ALL, cytotoxic chemotherapy	Yes	NS	Yes	AmB	NS	Death
52 [61]	71 years, F	Endophthalmic abscess[c]	…	NS	Trauma, keratitis, endophthalmitis	No	NS	NS	AmB then FLU, AmB irrigation	Surgery	Favorable
53 [62]	59 years, M	Esophagitis[c]	…	None	AIDS	No	NS	No	FLU[a,b]	NS	Favorable
54 [63]	40 years, M	Lung[c]	…	None	None, professional exposure as a baker	No	No	No	Surgery	NS	Favorable

NOTE. ALL, acute lymphoid leukemia; AmB, amphotericin B; AML, acute myeloid leukemia; ATB, previous antibiotic therapy; BMT, bone marrow transplantation; CAPD, chronic ambulatory peritoneal dialysis; CML, chronic myeloid leukemia; COPD, chronic obstructive pulmonary disease; CRD, chronic respiratory disease; CVC, central venous catheter; EBRA, erythroblastic refractory anemia; ECMO, extracorporeal membrane oxygenation; FLU, fluconazole; ITR, itraconazole; IVC, intravenous catheter; IVDA, injection drug addiction; KET, ketoconazole; MCZ, miconazole; NeutP, neutropenia; NS, not specified; VR, valvular replacement; 5-FC, 5-flucytosine.

[a] Prophylactic or curative treatment with an azole derivate at the time of *S. cerevisiae* isolation.

[b] Documented resistance to ITR and decreased susceptibility to FLU.

[c] Histologically proven.

of women) [12, 13], and *S. cerevisiae* is considered to be responsible for symptoms mimicking *Candida* vaginitis in 0.4% of cases [14, 15]. A more recent study involving patients with hematologic disease reported isolation of *S. cerevisiae* from throat, stool, urine, and perineum samples in 16%, 23%, 10%, and 20% of the patients, respectively [16]. It is not known whether *S. cerevisiae* is a persistent commensal of the digestive tract or whether it is only transiently present after food ingestion. Interestingly, among 70 patients hospitalized for bone marrow transplantation, *S. cerevisiae* was isolated only once, despite weekly surveillance cultures (primarily of stool and throat swab specimens) during hospitalization [17].

Since the 1980s, *S. cerevisiae* has also been isolated from persons with pathogenic conditions and has been considered to be a cause of invasive fungal infections. The incidence of *Saccharomyces* fungemia varied from 1% in a retrospective study of 102 nosocomial cases of fungemia [18] to 3.6% among patients at French teaching hospitals [9]. Among these cases, there is a growing number of observations regarding invasive infections with *S. boulardii*. To generate hypotheses for future evaluation of preventive strategies, we performed a comprehensive review of published reports of invasive *Saccharomyces* infections, with an emphasis on epidemiologic and clinical characteristics and the differences between *S. cerevisiae* and *S. boulardii* invasive infections.

MATERIALS AND METHODS

We reviewed published reports of invasive *Saccharomyces* infection found in the Medline database. The following key words were searched: "*Saccharomyces,*" "epidemiology," "invasive," "infection," "sepsis," and "fungemia." Reports published through May 2005 in English, French, German, or Spanish were reviewed. We only considered proven infection, based on the definitions of the Invasive Fungal Infection Cooperative Group of the European Organization for Research and Treatment of Cancer–Mycosis Study Group of the National Institute of Allergy and Infectious Diseases cooperative group [19]: fungemia, isolation from normally sterile fluids (e.g., pleural and synovial fluids), and deep-site infections either histologically proven for sites susceptible to colonization (lungs, peritoneum, esophagus) [20, 21]. We did not include asymptomatic patients with only 1 organism isolated from the peritoneal cavity after digestive perforation or surgery, or from urine, oral swab, stool, or vaginal swab specimens, because such patients were more likely to have colonization rather than true infection [20–22]. Patients with insufficient clinical information were excluded [7, 18].

We considered *S. boulardii* to be the etiologic agent if the patient received treatment with a probiotic containing *S. boulardii* and/or if a molecular typing method confirmed the identification of this pathogen. Data on age, sex, underlying disease, immunological status, previous antibiotic therapy, and intravenous catheter use were collected for each case. Immunodeficiency was considered when hematological malignancy, bone marrow transplantation, neoplasia, profound neutropenia, immunosuppressive therapy, or AIDS were reported. Profound neutropenia was defined as an absolute neutrophil count of $<0.5 \times 10^9$ neutrophils/L at the time of *Saccharomyces* isolation. Antibiotic therapy was taken into account as a possible predisposing factor when given for at least 1 week before the isolation of *Saccharomyces* organisms.

Antifungal treatment and removal of intravenous catheter were also recorded. The outcome was considered to be favorable if the patient survived or if the infection was cured (e.g., culture results after treatment were negative). We also investigated in vitro susceptibility by reviewing studies that included >10 isolates that underwent susceptibility testing by means of commercially available compounds.

Statistical analysis. Values are means ± SD unless otherwise specified. Comparisons between groups were performed using Fisher's exact test for continuous variables and a χ^2 test for percentages. P values of <.05 were considered to be statistically significant.

RESULTS

In a comprehensive review, we found 92 documented cases of proven invasive *Saccharomyces* infection. Fifteen cases (16.3%) were diagnosed before 1990, and 76 cases (82.6%) were diagnosed after 1990. The age of the participants was 44.3 ± 26.9 years; the youngest patient was 9 days old, and the oldest patient was 89 years old (tables 1 and 2). The sex ratio was 0.74 (35 females and 47 males). Patients with *S. cerevisiae* fungemia were significantly younger (age, 38.3 ± 27.5 vs. 53.8 ± 21.6 years; $P < .02$) and more likely to be female (sex ratio, 1.14 vs. 0.17; $P < .02$) than were patients with localized (i.e., single-organ) infection.

Underlying conditions. All patients had at least 1 condition facilitating the development of invasive fungal infection. Intravenous catheter use (by 47 patients) and previous receipt of antibiotic therapy (by 45 patients) were the most frequently reported predisposing factors (tables 1 and 2). *S. boulardii* was considered to be the etiologic agent in 37 cases. Molecular identification was performed in 23 cases, mainly by means of restriction-enzyme analysis of mitochondrial DNA (table 2). Among the 37 patients with presumed *S. boulardii* infection, 5 did not take a probiotic containing *S. boulardii* at the time of diagnosis.

Compared with patients infected with *S. cerevisiae,* patients infected with *S. boulardii* were more likely to have digestive tract disease (5.6% vs. 58.3%; $P < .001$), to have an intravenous catheter (29% vs. 83.8%; $P < .0001$), and to be hospitalized in

Table 2. Clinical and microbiologic features of 37 patients with *Saccharomyces boulardii* fungemia.

Patient, reference	Age, sex	Molecular diagnostic tool	Underlying disease	Immuno-deficiency	IVC	Previous antibiotic therapy	*S. boulardii* therapy	ATF	IVC removal	Outcome
1 [64]	33 years, M	NS	Ulcerative colitis, abdominal surgery, corticosteroid therapy	NS	Yes	Yes	Yes	AmB, FLU	NS	Favorable
2 [65]	14 years, M	NS	Burn	NS	Yes	Yes	Yes	AmB, 5-FC	Yes	Favorable
3 [66]	1 year, F	NS	Gastroenteritis, malnutrition	Yes	Yes	Yes	Yes	FLU	NS	Favorable
4 [67]	51 years, F	DNA fingerprinting	Polyarteritis nodosa, immunosuppressive therapy	NS	Yes	Yes	Yes	AmB	Yes	Favorable
5 [8]	49 years, M	PFGE karyotyping	Pneumonia	NS	Yes	Yes	Yes	FLU	NS	Favorable
6 [68]	78 years, F	NS	COPD, intestinal disease	NS	Yes	Yes	Yes	FLU	Yes	Favorable
7[a] [47]	8 months, M	ND	Acute myeloid leukemia	Yes	Yes	NS	Yes	AmB	Yes	Favorable
8 [5]	30 months, M	mtDNA REA	Coloileal anastomosis, esogastric surgery, cystic fibrosis, bacteremia	NS	Yes	Yes	Yes	AmB	Yes	Favorable
9 [5]	36 years, M	mtDNA REA	AIDS, cytotoxic chemotherapy for lymphoma	Yes	Yes	Yes	Yes	FLU	Yes	Favorable
10 [5]	47 years, M	mtDNA REA	Esophagus neoplasia, esophagectomy	NS	Yes	Yes	Yes	FLU	Yes	Favorable
11 [5]	78 years, F	mtDNA REA	ARDS, digestive hemorrhage	NS	Yes	Yes	Yes	None	No	Favorable
12 [5]	31 years, F	NS	AIDS	Yes	Yes	NS	Yes	AmB	NS	Favorable
13 [5]	36 years, F	NS	AIDS	Yes	Yes	NS	Yes	AmB	NS	Favorable
14 [5]	20 months, M	NS	Intestinal atresia	No	Yes	NS	Yes	NS	NS	NS
15 [69]	3 months, M	mtDNA REA	Surgery for congenital cardiopathy	NS	Yes	Yes	Yes	AmB	Yes	Favorable
16 [69]	1 month, F	mtDNA REA	Surgery for intestinal atresia	NS	Yes	Yes	No	AmB	Yes	Favorable
17[a] [70]	74 years, M	ND	Subarachnoidial hematoma, neurosurgery	No	Yes	NS	Yes	FLU	NS	Death
18 [6]	50 years, M	nDNA plus mtDNA REA	Cardiac arrest	No	Yes	NS	Yes	None	NS	Death
19 [6]	51 years, F	nDNA plus mtDNA REA	Aortic surgery, cachexia	Yes	Yes	NS	Yes	FLU	NS	Death
20 [6]	50 years, M	nDNA plus mtDNA REA	ARDS, gastric ulcer	No	Yes	NS	No	FLU	Yes	Favorable
21 [6]	82 years, F	nDNA plus mtDNA REA	Acute respiratory failure	NS	Yes	NS	Yes	None	NS	Favorable
22 [6]	75 years, M	nDNA plus mtDNA REA	Acute respiratory failure	NS	Yes	NS	Yes	None	NS	Favorable
23 [6]	77 years, M	nDNA plus mtDNA REA	Peritonitis, duodenal ulcer	Yes	Yes	NS	Yes	AmB	NS	Death
24 [6]	71 years, F	nDNA plus mtDNA REA	Hemorrhagic CVS	Yes	Yes	NS	Yes	None	NS	Favorable
25 [7]	34 years, M	PFGE karyotyping	Head and thoracic trauma	No	Yes	Yes	No	FLU	No	Favorable
26 [7]	48 years, M	PFGE karyotyping	Rupture of cerebral aneurism	No	Yes	Yes	No	FLU	Yes	Favorable
27 [7]	75 years, F	PFGE karyotyping	Myocardial infraction	No	Yes	Yes	No	FLU	Yes	Favorable
28 [71]	48 years, M	NS	Diabetes, femoropopliteal bypass	No	NS	Yes	Yes	None	NS	Death
29[a] [72]	3 weeks, M	ND	Premature birth, intestinal disease	No	Yes	NS	Yes	AmB	Yes	Favorable
30 [73]	42 years, F	PFGE karyotyping plus RFLP	Kidney-pancreas transplantation, cyclosporine, *C. difficile* diarrhea	NS	NS	Yes	Yes	FLU	NS	Favorable
31 [73]	41 years, M	PFGE karyotyping plus RFLP	AIDS	NS	NS	Yes	Yes	AmB	NS	Favorable
32 [74]	89 years, F	ND	Intestinal disease	No	No	Yes	Yes	FLU	No	Death
33 [75]	65 years, M	ND	Head and neck cancer, intestinal disease	Yes	Yes	Yes	Yes	AmB	Yes	Favorable
34 [76]	19 years, M	ND	Spastic tetraparesis, gastrostomy	No	NS	No	Yes	FLU,[b] then VRZ	NS	Favorable
35[77]	76 years, F	DNA fingerprinting	Diabetes, heart surgery	No	Yes	Yes	Yes	FLU	NS	Death
36 [77]	72 years, F	DNA fingerprinting	Heart surgery	No	Yes	Yes	Yes	None	NS	Death
37 [77]	74 years, F	DNA fingerprinting	Rheumatoid arthritis, corticosteroid therapy, heart surgery	NS	No	Yes	Yes	FLU	NS	Death

NOTE. AmB, amphotericin B; ARDS, acute respiratory distress syndrome; COPD, chronic obstructive pulmonary disease; CVS, cerebral vascular stroke; FLU, fluconazole; ND, not done; nDNA, nuclear DNA; NS, not specified; REA, restriction endonuclease analysis; RFLP, restriction fragment–length polymorphism; VRZ, voriconazole; 5-FC, 5-flucytosine.

[a] Diagnosed as *S. cerevisiae* with phenotypic methods.

[b] Administered for 7 days.

Table 3. *Saccharomyces cerevisiae* antifungal susceptibilities in studies in which >10 isolates were tested.

Antifungal, reference(s)	MIC range, mg/L	MIC_{50}, mg/L	MIC_{90}, mg/L	No. of isolates
Amphotericin B				
[17]	0.12–2	1	1	74
[78, 79]	0.5–1	1	1	22
[80]	0.125–1	1	1	24
[81]	0.25–4	1	1	30
[81][a]	0.25–1	0.5	1	30
[82]	0.25–0.5	0.25	0.25	11
[16]	0.25–4	0.5	1	160
[83][b]	0.032–1	0.5	ND	104
5-Fluorocytosine				
[17]	0.25–32	0.25	0.25	74
[78, 79]	0.06–0.12	0.06	0.12	22
[80]	≤0.0313 to 0.25	0.0625	0.125	24
[81]	≤0.125 to 1	≤0.125	1	30
[81][a]	≤0.125 to 0.5	≤0.125	≤0.125	30
Fluconazole				
[17]	0.12–16	2	8	74
[78, 79]	0.5–16	2	16	22
[80]	≤0.0313 to 4	0.5	2	24
[81]	0.5–16	2	4	30
[81][a]	0.5–16	8	8	30
[82]	0.5–8	2	4	11
[16]	1–128	64	128	160
[83][b]	2–64	32	ND	104
Itraconazole				
[17]	0.015–1	0.5	1	74
[78, 79]	0.03–0.5	0.5	0.5	22
[80]	≤0.0313 to 4	0.25	2	24
[81]	≤0.007 to 0.5	0.125	0.25	30
[81][a]	0.125–2	1	1	30
[82]	0.064–0.25	0.063	0.25	11
[16]	0.25–16	8	16	160
[83][b]	1–64	64	ND	104
Ketoconazole				
[80]	≤0.0313 to 2	0.25	1	24
[81]	≤0.03 to 1	0.25	0.5	30
[81][a]	0.03–1	0.5	1	30
Voriconazole				
[82]	≤0.008 to 8	0.032	0.125	11
[83][b]	0.016–2	0.125	ND	104

NOTE. The majority of in vitro activities of antifungal agents were determined using the microdilution method (NCCLS) with Roswell Park Memorial Institute 1640 medium. ND, not done.

[a] Antifungal in vitro activities were determined using the microdilution method (NCCLS) with yeast nitrogen base medium.

[b] Antifungal in vitro activities performed using Etest (AB Biodisk) and/or the microdilution method (NCCLS).

an intensive care unit (0.05% vs. 32.4%; $P < .01$). In contrast, the frequency of immunocompromise was lower among patients infected with *S. boulardii* than among patients infected with *S. cerevisiae* (25% vs. 58.5%; $P < .01$).

Involved sites. *Saccharomyces* organisms were most frequently isolated from blood (72 patients), either exclusively (63 patients) or with involvement of other organ(s) (9 patients) (table 1). *S. boulardii* was exclusively isolated from blood ($n = 37$). Concomitant isolation of *S. cerevisiae* and a second microorganism, mainly enterobacteria, was reported in 7 cases of fungemia. Patient 10 received a postmortem diagnosis of disseminated infection, although positive blood culture results were not recorded before death. In the remaining 17 cases, the infection was localized to a single organ. Overall, the main sites were the lungs (for 8 patients) and the heart valves (for 6 patients).

Treatment and outcome. Therapy was specified for 81 patients (tables 1 and 2). Of these patients, 64 received antifungal therapy, with intravenous amphotericin B alone (31 patients) or in combination with other agents (7 patients) and fluconazole (19 patients) being the most frequently administered first-line therapy. At the time of *S. cerevisiae* isolation, 6 patients were receiving antifungal therapy: 4 were receiving fluconazole prophylaxis, and 2 were receiving curative treatment with ketoconazole or itraconazole. Of the 32 patients for whom action regarding the catheter was reported, 27 had had their catheter removed.

Outcome was reported in 84 cases and was considered to be favorable in 57 (68.7%). Patients with *S. boulardii* infection had a better prognosis than patients with *S. cerevisiae* infection (73% vs. 55.5%; $P < .01$). The rates of favorable outcome among immunocompromised patients (65.5%) and immunocompetent patients (73.8%) did not differ significantly. There was no significant difference in the rates of favorable outcome between patients treated with intravenous amphotericin B (77.7%) and patients treated with fluconazole (60%).

Results of in vitro susceptibility tests. We found 9 studies in which >10 isolates underwent antifungal susceptibility testing. The results are reported in table 3.

DISCUSSION

On the basis of data revealed in our literature search, invasive *Saccharomyces* infections remain rare among invasive fungal infections, although the incidence has significantly increased since the 1990s. Risk factors associated with invasive *Saccharomyces* infections are similar to those reported elsewhere for invasive candidiasis, except for treatment with a probiotic containing *S. boulardii*. It is important to emphasize the role of this biotherapeutic agent, because it was responsible for 40.2% of invasive *Saccharomyces* infections reported in the literature. In the 5 cases in which *S. boulardii* was considered to be the etiologic agent, although these patients did not take a probiotic preparation, the similarity of the genotypic profile between *S. boulardii* isolates from patients and from probiotic preparations strongly suggested nosocomial acquisition, with catheters being

a likely portal of entry because of possible contamination through hand transmission [6, 7].

The epidemiologic characteristics of *S. cerevisiae* infection in human beings is not fully understood. Persistent digestive implantation in healthy volunteers has never been described, suggesting that *S. cerevisiae* behaves mainly as a transient digestive commensal linked to contaminated food. Thus, the portal of entry for invasive infections is mainly supposed to be digestive. Evidence suggests that nosocomial acquisition of *S. cerevisiae* can occur, because clusters of identical isolates from patients concurrently hospitalized in the same unit have been described [17]. This is also supported by the increasing rates of colonization among hospitalized patients with hematologic disease who only ingest sterile food not comprised of raw fruits or vegetables [16]. The same conclusion could be drawn for *S. boulardii* infection in patients who did not receive treatment with a probiotic containing *S. boulardii,* with intravenous catheters the likely portal of entry [5]. Hand transmission has been suggested in these latter cases [5].

Saccharomyces infection is clinically indistinguishable from invasive candidiasis, notably because chorioretinitis (fluffy yellow exudates) and esophagitis (yellow-white plaques on an erythematous background) are present in both conditions, and is even indistinguishable from infections due to other microorganisms, such as endocarditis. In addition, patients have nonspecific symptoms that could be related to the underlying disease, and the diagnosis of *Saccharomyces* infection is often unexpected. Fever, when noted, is present in 75.2% of patients. However, the clinical impact of *Saccharomyces* infection has been clearly assessed in an immunocompetent patient whose unique predisposing factor was the ingestion of health food containing viable yeasts [25]. The patient developed recurrent fever, malaise with nausea, and night sweats. Yeasts were isolated from bone marrow and urine specimens. The patient recovered when he stopped ingesting health food containing yeasts. The occurrence of deep-site involvement with histologic documentation also underlines the pathogenicity of such yeasts. In these cases, yeasts were most often associated with necrosis [60] and granulomatous reaction [25].

Significant particularities of *S. boulardii* infections include higher frequency of intensive care unit hospitalization, presence of an indwelling catheter, and intestinal disease. This may either correspond to specific predisposing factors or, more likely, may reflect conditions associated with treatment with a probiotic containing *S. boulardii,* such as severe digestive disease. The lack of organ involvement and the better prognosis could be due to decreased *S. boulardii* virulence. Another explanation could be the ability of patients at risk to partly control the infection, given the lower frequency of immunocompromise in this group. *S. boulardii* showed an intermediate level of virulence in an experimental murine model of invasive infection involving both immunocompetent and immunocompromised mice [3].

S. cerevisiae has, in immunocompromised mice, been shown to grow at 42°C and to form pseudohyphae [84]. Also, *SSD1,* which encodes a protein that affects various cellular processes (including maintenance of cell integrity, control of cell cycle, and growth at high temperature), seems to play an important role in the virulence of *Saccharomyces* organisms [85]. Deletion of this gene leads to a significant increase in virulence for both clinical and plant isolates, as revealed in a mouse model of invasive infection [85]. Alteration in composition and cell wall architecture is supposed to lead to overstimulation of the proinflammatory response. Furthermore, analysis of feral and clinical strains of *S. cerevisiae* showed similar virulence in a mouse model of infection [85]. Similar to many opportunistic organisms, it is not possible to identify particular features of *S. cerevisiae* that distinguish between environmental and clinical strains on the basis of virulence-related phenotype [84].

S. cerevisiae exhibits low susceptibility to amphotericin B and to azole derivatives (table 3). Although breakpoints have not been defined for *S. cerevisiae*, MIC_{90} for fluconazole and itraconazole are considered to be within the susceptible dose-dependent range defined for *C. albicans* [86]. One can hypothesize that these drugs may play a role in the emergence of *S. cerevisiae.* Although data remain scarce, voriconazole seems to exhibit good efficacy against *S. cerevisiae,* with an MIC_{90} of <0.25 mg/L. A recent publication reports on the successful outcome with voriconazole therapy for a patient with *S. boulardii* who did not respond to initial treatment with fluconazole [76]. No published series is available for caspofungin treatment, but preliminary research has demonstrated good efficacy against a limited number of strains (C.H., unpublished data). MIC values for amphotericin B and fluconazole were 0.19 and 32 mg/L, respectively, as determined for 2 clinical isolates of *S. boulardii* [5]. *S. boulardii* could not be tested by Etest (AB Biodisk) on casitone or Roswell Park Memorial Institute 1640 agar because of the lack of growth on these media (A.E.-A. and C.H., unpublished data).

Despite this spectrum of susceptibility, a favorable outcome was observed in 63% of cases revealed in our literature search, which is a slightly higher percentage than that reported for cases of invasive candidiasis [87]. Specific data on morbidity were difficult to evaluate, because *Saccharomyces* infections often occurred in patients with a large number of comorbidities. Use of amphotericin B or fluconazole therapy with central venous catheter removal appeared to be an effective therapeutic option for patients with *Saccharomyces* infection. Favorable outcome may also be related to the low virulence of this species, a hypothesis that is supported by the absence of significant difference in outcome between immunocompromised and immunocompetent patients.

In conclusion, *Saccharomyces* organisms are increasingly reported as agents of invasive infection, especially in immunocompromised or critically ill patients. Special caution has to be taken with *S. boulardii* probiotic preparations, which account for nearly 40% of infection cases.

Acknowledgments

We thank members of the Unité de Génétique Moléculaire des Levures from the Paris Institut Pasteur; our laboratory members, for fruitful discussions; and Dr. Denis Angoulvant (INSERM E226 Université Cl. Bernard Lyon 1) and Cécile Fairhead (Paris Institut Pasteur), for their helpful reading and advice.

Potential conflicts of interest. A.A.-E. and C.H.: no conflicts.

References

1. Kwon-Chung KJ, Bennett JE. Medical mycology. Philadelphia: Lea & Febiger, **1992**.
2. Marteau PR, de Vrese M, Cellier CJ, Schrezenmeir J. Protection from gastrointestinal disease with the use of probiotics. Am J Clin Nutr **2001**; 73(Suppl 2):430S–6S.
3. McCullough MJ, Clemons KV, McCusker JH, Stevens DA. Species identification and virulence attributes of *Saccharomyces boulardii* (nom. inval.). J Clin Microbiol **1998**; 36:2613–7.
4. Mallie M, Nguyen V-P, Bertout S, Vaillant C, Bastide JM. Genotyping study of *Saccharomyces boulardii* compared to the *Saccharomyces sensu stricto* complex species. J Mycol Med **2001**; 11:19–25.
5. Hennequin C, Kauffmann-Lacroix C, Jobert A, et al. Possible role of catheters in *Saccharomyces boulardii* fungemia. Eur J Clin Microbiol Infect Dis **2000**; 19:16–20.
6. Lherm T, Monet C, Nougiere B, et al. Seven cases of fungemia with *Saccharomyces boulardii* in critically ill patients. Intensive Care Med **2002**; 28:797–801.
7. Cassone M, Serra P, Mondello F, et al. Outbreak of *Saccharomyces cerevisiae* subtype *boulardii* fungemia in patients neighboring those treated with a probiotic preparation of the organism. J Clin Microbiol **2003**; 41:5340–3.
8. Fredenucci I, Chomarat M, Boucaud C, Flandrois JP. *Saccharomyces boulardii* fungemia in a patient receiving Ultra-levure therapy. Clin Infect Dis **1998**; 27:222–3.
9. Piarroux R, Millon L, Bardonnet K, Vagner O, Koenig H. Are live *Saccharomyces* yeasts harmful to patients? Lancet **1999**; 353:1851–2.
10. Hennequin C, Thierry A, Richard GF, et al. Microsatellite typing as a new tool for identification of *Saccharomyces cerevisiae* strains. J Clin Microbiol **2001**; 39:551–9.
11. Greer A, Gemoets H. The coexistence of pathogenic fungi in certain chronic pulmonary diseases: with especial reference to pulmonary tuberculosis. Dis Chest **1943**; 9:212–24.
12. Erdem H, Cetin M, Timuroglu T, Cetin A, Yanar O, Pahsa A. Identification of yeasts in public hospital primary care patients with or without clinical vaginitis. Aust N Z J Obstet Gynaecol **2003**; 43:312–6.
13. Posteraro B, Sanguinetti M, D'Amore G, Masucci L, Morace G, Fadda G. Molecular and epidemiological characterization of vaginal *Saccharomyces cerevisiae* isolates. J Clin Microbiol **1999**; 37:2230–5.
14. McCullough MJ, Clemons KV, Farina C, McCusker JH, Stevens DA. Epidemiological investigation of vaginal *Saccharomyces cerevisiae* isolates by a genotypic method. J Clin Microbiol **1998**; 36:557–62.
15. Sobel JD, Vazquez J, Lynch M, Meriwether C, Zervos MJ. Vaginitis due to *Saccharomyces cerevisiae:* epidemiology, clinical aspects, and therapy. Clin Infect Dis **1993**; 16:93–9.
16. Salonen JH, Richardson MD, Gallacher K, et al. Fungal colonization of haematological patients receiving cytotoxic chemotherapy: emergence of azole-resistant *Saccharomyces cerevisiae*. J Hosp Infect **2000**; 45:293–301.
17. Zerva L, Hollis RJ, Pfaller MA. In vitro susceptibility testing and DNA typing of *Saccharomyces cerevisiae* clinical isolates. J Clin Microbiol **1996**; 34:3031–4.
18. Taylor GD, Buchanan-Chell M, Kirkland T, McKenzie M, Wiens R. Trends and sources of nosocomial fungaemia. Mycoses **1994**; 37: 187–90.
19. Ascioglu S, Rex JH, de Pauw B, et al. Defining opportunistic invasive fungal infections in immunocompromised patients with cancer and hematopoietic stem cell transplants: an international consensus. Invasive Fungal Infections Cooperative Group of the European Organization for Research and Treatment of Cancer; Mycoses Study Group of the National Institute of Allergy and Infectious Diseases. Clin Infect Dis **2002**; 34:7–14.
20. Eng RH, Drehmel R, Smith SM, Goldstein EJ. *Saccharomyces cerevisiae* infections in man. Sabouraudia **1984**; 22:403–7.
21. Olver WJ, James SA, Lennard A, et al. Nosocomial transmission of *Saccharomyces cerevisiae* in bone marrow transplant patients. J Hosp Infect **2002**; 52:268–72.
22. Tiballi RN, Spiegel JE, Zarins LT, Kauffman CA. *Saccharomyces cerevisiae* infections and antifungal susceptibility studies by colorimetric and broth macrodilution methods. Diagn Microbiol Infect Dis **1995**; 23:135–40.
23. Stein PD, Folkens AT, Hruska KA. *Saccharomyces* fungemia. Chest **1970**; 58:173–5.
24. Rubinstein E, Noriega ER, Simberkoff MS, Holzman R, Rahal JJ Jr. Fungal endocarditis: analysis of 24 cases and review of the literature. Medicine (Baltimore) **1975**; 54:331–4.
25. Jensen DP, Smith DL. Fever of unknown origin secondary to brewer's yeast ingestion. Arch Intern Med **1976**; 136:332–3.
26. Eschete ML, West BC. *Saccharomyces cerevisiae* septicemia. Arch Intern Med **1980**; 140:1539.
27. Cimolai N, Gill MJ, Church D. *Saccharomyces cerevisiae* fungemia: case report and review of the literature. Diagn Microbiol Infect Dis **1987**; 8:113–7.
28. Sethi N, Mandell W. *Saccharomyces fungemia* in a patient with AIDS. N Y State J Med **1988**; 88:278–9.
29. Anaissie E, Bodey GP, Kantarjian H, et al. New spectrum of fungal infections in patients with cancer. Rev Infect Dis **1989**; 11:369–78.
30. Manzella JP, Shaffer S, Agarwal N, Kellogg JA. *Saccharomyces cerevisiae* fungemia in a multiply traumatized patient. J Trauma **1989**; 29:129–30.
31. Tawfik OW, Papasian CJ, Dixon AY, Potter LM. *Saccharomyces cerevisiae* pneumonia in a patient with acquired immune deficiency syndrome. J Clin Microbiol **1989**; 27:1689–91.
32. Nielsen H, Stenderup J, Bruun B. Fungemia with Saccharomycetaceae: report of four cases and review of the literature. Scand J Infect Dis **1990**; 22:581–4.
33. Aucott JN, Fayen J, Grossnicklas H, Morrissey A, Lederman MM, Salata RA. Invasive infection with *Saccharomyces cerevisiae:* report of three cases and review. Rev Infect Dis **1990**; 12:406–11.
34. Bonnay S, Darchis JP, Veyssier P. *Saccharomyces cerevisae* septicaemia in a cancer patient. Médecine et Maladies infectieuses **1991**; 21:32–4.
35. Oriol A, Ribera JM, Arnal J, Milla F, Batlle M, Feliu E. *Saccharomyces cerevisiae* septicemia in a patient with myelodysplastic syndrome [letter]. Am J Hematol **1993**; 43:325–6.
36. Cairoli R, Marenco P, Perego R, de Cataldo F. *Saccharomyces cerevisiae* fungemia with granulomas in the bone marrow in a patient undergoing BMT. Bone Marrow Transplant **1995**; 15:785–6.
37. Muehrcke DD, Lytle BW, Cosgrove DM 3rd. Surgical and long-term antifungal therapy for fungal prosthetic valve endocarditis. Ann Thorac Surg **1995**; 60:538–43.
38. Fung KS, Scheel O, Lyon DJ, Cheng AF, Bendeck J. Self-inflicted bacteraemia and fungaemia in Vietnamese migrants. Scand J Infect Dis **1996**; 28:83–5.
39. Debelian GJ, Olsen I, Tronstad L. Observation of *Saccharomyces cer-*

evisiae in blood of patient undergoing root canal treatment. Int Endod J **1997**; 30:313–7.
40. Eholie SP, N'Gbocho L, Bissagnene E, et al. Profound mycoses in AIDS in Abidjan (Cote d'Ivoire) [in French]. Bull Soc Pathol Exot **1997**; 90: 307–11.
41. Yoshida M, Obayashi T, Iwama A, et al. Detection of plasma (1→3)-*β*-D-glucan in patients with *Fusarium, Trichosporon, Saccharomyces* and *Acremonium* fungaemias. J Med Vet Mycol **1997**; 35:371–4.
42. Sparrelid E, Hagglund H, Remberger M, et al. Bacteraemia during the aplastic phase after allogeneic bone marrow transplantation is associated with early death from invasive fungal infection. Bone Marrow Transplant **1998**; 22:795–800.
43. Fiore NF, Conway JH, West KW, Kleiman MB. *Saccharomyces cerevisiae* infections in children. Pediatr Infect Dis J **1998**; 17:1177–9.
44. Pavese P, Brion JP, Lebeau B, Grillot R, Ambroise-Thomas P. Epidemiology of fungemia in a university hospital; therapeutic incidence [in French]. Pathol Biol (Paris) **1999**; 47:579–83.
45. Heath CH, Jaksic A, McKerracher D, Clarke GM. Disseminated *Saccharomyces cerevisiae* infection following polymicrobial hepatobiliary sepsis [letter]. Aust N Z J Med **2000**; 30:521–2.
46. Ramirez Moreno A, Anguita Sanchez M, Castillo Dominguez JC, Siles Rubio JR, Torres Calvo F, Valles Belsue F. Fungal endocarditis in non drug-addict patients. 10-year experience [in Spanish]. Rev Esp Cardiol **2000**; 53:507–10.
47. Cesaro S, Chinello P, Rossi L, Zanesco L. *Saccharomyces cerevisiae* fungemia in a neutropenic patient treated with *Saccharomyces boulardii*. Support Care Cancer **2000**; 8:504–5.
48. Ipson MA, Blanco CL. *Saccharomyces cerevisiae* sepsis in a 35-week-old premature infant: a case report. J Perinatol **2001**; 21:459–60.
49. Morrison VA, Haake RJ, Weisdorf DJ. The spectrum of non-*Candida* fungal infections following bone marrow transplantation. Medicine (Baltimore) **1993**; 72:78–89.
50. Ubeda P, Perez-Belles C, Blanes M, Viudes A, Peman J, Gobernado M. Infective fungal endocarditis [in Spanish]. Enferm Infecc Microbiol Clin **2001**; 19:500–2.
51. Smith D, Metzgar D, Wills C, Fierer J. Fatal *Saccharomyces cerevisiae* aortic graft infection. J Clin Microbiol **2002**; 40:2691–2.
52. Ruiz-Esquide F, Diaz MC, Wu E, Silva V. Verrucous endocarditis secondary to *Saccharomyces cerevisiae*: a case report [in Spanish]. Rev Med Chil **2002**; 130:1165–9.
53. Feld R, Fornasier VL, Bombardier C, Hastings DE. Septic arthritis due to *Saccharomyces species* in a patient with chronic rheumatoid arthritis. J Rheumatol **1982**; 9:637–40.
54. Dougherty SH, Simmons RL. Postoperative peritonitis caused by *Saccharomyces cerevisiae*. Arch Surg **1982**; 117:248.
55. Rippon JW. Medical mycology: the pathogenic fungi and the pathogenic *Actinomycetes*. 3rd ed. Philadelphia: Saunders, **1988**.
56. Doyle MG, Pickering LK, O'Brien N, Hoots K, Benson JE. *Saccharomyces cerevisiae* infection in a patient with acquired immunodeficiency syndrome. Pediatr Infect Dis J **1990**; 9:850–1.
57. Chertow GM, Marcantonio ER, Wells RG. *Saccharomyces cerevisiae* empyema in a patient with esophago-pleural fistula complicating variceal sclerotherapy. Chest **1991**; 99:1518–9.
58. Snyder S. Peritonitis due to *Saccharomyces cerevisiae* in a patient on CAPD. Perit Dial Int **1992**; 12:77–8.
59. Senneville E, Ajana F, Gerard Y, et al. Bilateral ureteral obstruction due to *Saccharomyces cerevisiae* fungus balls. Clin Infect Dis **1996**; 23:636–7.
60. Hovi L, Saarinen UM, Donner U, Lindqvist C. Opportunistic osteomyelitis in the jaws of children on immunosuppressive chemotherapy. J Pediatr Hematol Oncol **1996**; 18:90–4.
61. Kirsch LS, Brownstein S, Deschenes J, Sorgini C, Jackson WB. *Saccharomyces* keratitis and endophthalmitis. Can J Ophthalmol **1999**; 34: 229–32.
62. Konecny P, Drummond FM, Tish KN, Tapsall JW. *Saccharomyces cerevisiae* oesophagitis in an HIV-infected patient. Int J STD AIDS **1999**; 10:821–2.
63. Ren P, Sridhar S, Chaturvedi V. Use of paraffin-embedded tissue for identification of *Saccharomyces cerevisiae* in a baker's lung nodule by fungal PCR and nucleotide sequencing. J Clin Microbiol **2004**; 42: 2840–2.
64. Zunic P, Lacotte J, Pegoix M, et al. *Saccharomyces boulardii* fungemia: apropos of a case [in French]. Therapie **1991**; 46:498–9.
65. Viggiano M, Badetti C, Bernini V, Garabedian M, Manelli JC. *Saccharomyces boulardii* fungemia in a patient with severe burns [in French]. Ann Fr Anesth Reanim **1995**; 14:356–8.
66. Pletincx M, Legein J, Vandenplas Y. Fungemia with *Saccharomyces boulardii* in a 1-year-old girl with protracted diarrhea. J Pediatr Gastroenterol Nutr **1995**; 21:113–5.
67. Bassetti S, Frei R, Zimmerli W. Fungemia with *Saccharomyces cerevisiae* after treatment with *Saccharomyces boulardii*. Am J Med **1998**; 105: 71–2.
68. Niault M, Thomas F, Prost J, Ansari FH, Kalfon P. Fungemia due to *Saccharomyces species* in a patient treated with enteral *Saccharomyces boulardii*. Clin Infect Dis **1999**; 28:930.
69. Perapoch J, Planes AM, Querol A, et al. Fungemia with *Saccharomyces cerevisiae* in two newborns, only one of whom had been treated with ultra-levura. Eur J Clin Microbiol Infect Dis **2000**; 19:468–70.
70. Rijnders BJ, Van Wijngaerden E, Verwaest C, Peetermans WE. *Saccharomyces* fungemia complicating *Saccharomyces boulardii* treatment in a non-immunocompromised host. Intensive Care Med **2000**; 26:825.
71. Lestin F, Pertschy A, Rimek D. Fungemia after oral treatment with *Saccharomyces boulardii* in a patient with multiple comorbidities [in German]. Dtsch Med Wochenschr **2003**; 128:2531–3.
72. Lungarotti MS, Mezzetti D, Radicioni M. Methaemoglobinaemia with concurrent blood isolation of *Saccharomyces* and *Candida*. Arch Dis Child Fetal Neonatal Ed **2003**; 88:F446.
73. Riquelme AJ, Calvo MA, Guzman AM, et al. *Saccharomyces cerevisiae* fungemia after *Saccharomyces boulardii* treatment in immunocompromised patients. J Clin Gastroenterol **2003**; 36:41–3.
74. Cherifi S, Robberecht J, Miendje Y. *Saccharomyces cerevisiae* fungemia in an elderly patient with *Clostridium difficile* colitis. Acta Clin Belg **2004**; 59:223–4.
75. Henry S, D'Hondt L, Andre M, Holemans X, Canon JL. *Saccharomyces cerevisiae* fungemia in a head and neck cancer patient: a case report and review of the literature. Acta Clin Belg **2004**; 59:220–2.
76. Burkhardt O, Kohnlein T, Pletz M, Welte T. *Saccharomyces boulardii* induced sepsis: successful therapy with voriconazole after treatment failure with fluconazole. Scand J Infect Dis **2005**; 37:69–72.
77. Munoz P, Bouza E, Cuenca-Estrella M, et al. *Saccharomyces cerevisiae* fungemia: an emerging infectious disease. Clin Infect Dis **2005**; 40: 1625–34.
78. Pfaller MA, Messer S, Jones RN. Activity of a new triazole, Sch 56592, compared with those of four other antifungal agents tested against clinical isolates of *Candida* spp. and *Saccharomyces cerevisiae*. Antimicrob Agents Chemother **1997**; 41:233–5.
79. Pfaller MA, Messer SA, Coffman S. In vitro susceptibilities of clinical yeast isolates to a new echinocandin derivative, LY303366, and other antifungal agents. Antimicrob Agents Chemother **1997**; 41:763–6.
80. Zhanel GG, Karlowsky JA, Zelenitsky SA, Turik MA, Hoban DJ. Susceptibilities of *Candida* species isolated from the lower gastrointestinal tracts of high-risk patients to the new semisynthetic echinocandin LY303366 and other antifungal agents. Antimicrob Agents Chemother **1998**; 42:2446–8.
81. Barchiesi F, Arzeni D, Compagnucci P, Di Francesco LF, Giacometti A, Scalise G. In vitro activity of five antifungal agents against clinical isolates of *Saccharomyces cerevisiae*. Med Mycol **1998**; 36:437–40.
82. Swinne D, Watelle M, Van der Flaes M, Nolard N. In vitro activities of voriconazole (UK-109, 496), fluconazole, itraconazole and amphotericin B against 132 non-*albicans* bloodstream yeast isolates (CANARI study). Mycoses **2004**; 47:177–83.
83. Pelaez T, Perez-Olaso O, Garcia-Escribano N, et al. Saccharomyces cerevisiae: epidemiology and in vitro susceptibility testing in a general hospital over an 18- year period. In: Proceedings of the 44th Interscience Conference on Antimicrobial Agents and Chemotherapy

(Washington, D.C.). Washington, D.C.: American Society for Microbiology, **2004**:422–3.
84. McCusker JH, Clemons KV, Stevens DA, Davis RW. *Saccharomyces cerevisiae* virulence phenotype as determined with CD-1 mice is associated with the ability to grow at 42 degrees C and form pseudohyphae. Infect Immun **1994**; 62:5447–55.
85. Wheeler RT, Kupiec M, Magnelli P, Abeijon C, Fink GR. A *Saccharomyces cerevisiae* mutant with increased virulence. Proc Natl Acad Sci U S A **2003**; 100:2766–70.
86. Rex JH, Pfaller MA, Galgiani JN, et al. Development of interpretive breakpoints for antifungal susceptibility testing: conceptual framework and analysis of in vitro–in vivo correlation data for fluconazole, itraconazole, and candida infections: Subcommittee on Antifungal Susceptibility Testing of the National Committee for Clinical Laboratory Standards. Clin Infect Dis **1997**; 24:235–47.
87. Viscoli C, Girmenia C, Marinus A, et al. Candidemia in cancer patients: a prospective, multicenter surveillance study by the Invasive Fungal Infection Group (IFIG) of the European Organization for Research and Treatment of Cancer (EORTC). Clin Infect Dis **1999**; 28:1071–9.

SUPPLEMENT ARTICLE

Clinical Indications for Probiotics: An Overview

B. R. Goldin and S. L. Gorbach
Tufts University School of Medicine, Boston, Massachusetts

Probiotic bacteria are used to treat or prevent a broad range of human diseases, conditions, and syndromes. In addition, there are areas of medical use that have been proposed for future probiotic applications. Randomized double-blind studies have provided evidence of probiotic effectiveness for the treatment and prevention of acute diarrhea and antibiotic-induced diarrhea, as well as for the prevention of cow milk–induced food allergy in infants and young children. Research studies have also provided evidence of effectiveness for the prevention of traveler's diarrhea, relapsing *Clostridium difficile*–induced colitis, and urinary tract infections. There are also studies indicating that probiotics may be useful for prevention of respiratory infections in children, dental caries, irritable bowel syndrome, and inflammatory bowel disease. Areas of future interest for the application of probiotics include colon and bladder cancers, diabetes, and rheumatoid arthritis. The probiotics with the greatest number of proven benefits are *Lactobacillus rhamnosus* strain GG and *Saccharomyces boulardii.*

Probiotics have been defined as "live microorganisms which when administered in adequate amounts confer a health benefit on the host" [1]. Probiotics have been used to treat a wide range of diseases, ailments, and conditions that affect humans and animals. Additional medical applications have been proposed for potential future uses, depending on the outcomes of future experimental studies. The clinical uses of probiotics are broad; however, the clinical indications based on evidence-based studies are much narrower and are open to continuing evaluation. Table 1 contains a partial list of human diseases and conditions that probiotics have been used to prevent and/or treat.

DOCUMENTATION OF THE HEALTH EFFECTS OF PROBIOTICS FOR HUMAN DISEASES AND DISORDERS

Lactose malabsorption. A large number of people, as they age, experience a decline in the level of lactase (β-galactosidase) in the intestinal brush border mucosa. This decline causes lactose to be incompletely absorbed, resulting in flatus, bloating, abdominal cramps, and moderate-to-severe (watery) diarrhea. This results in a severe limitation in consumption of dairy products among the elderly population. There have been several studies that have demonstrated that, during the fermentative process involved in the production of yogurt, lactase is produced, which can exert its influence in the intestinal tract [2–5]. The organisms commonly used for the production of yogurt are *Lactobacillus bulgaricus* and *Streptococcus salivarius* subsp. *thermophilus*. Kim and Gilliland [4] found that feeding lactose-intolerant individuals yogurt caused a significant reduction in the level of breath hydrogen compared with that in subjects who were fed milk. The level of hydrogen in the breath is an indication of the extent of lactose metabolism in the large bowel. Kolars et al. [5] observed that the ingestion of 18 g of lactose in yogurt caused the production of 67% less hydrogen in the breath compared with that produced by a similar dose of lactose delivered in milk. Analysis of aspirates obtained from the duodenum 1 h after the consumption of yogurt showed significant levels of lactase [5]. These studies indicate that the delivery of lactase to the intestine via the consumption of lactase-producing probiotics is a practical approach for treatment of lactose malabsorption.

Acute diarrhea. There are at least 12 studies that have reported the use of probiotics to either treat or prevent acute diarrhea [6–17]. The majority of these

Reprints or correspondence: Dr. Barry R. Goldin, Tufts University School of Medicine, 136 Harrison Ave., Boston, MA 02111 (barry.goldin@tufts.edu).

Clinical Infectious Diseases 2008; 46:S96–100

1058-4838/2008/4603S2-0010$15.00
DOI: 10.1086/523333

Table 1. Medical applications in humans for different classes of probiotics.

Medical condition	Class(es) of probiotic	Reference(s)
Lactose maldigestion	LAB and *Streptococcus salivarius* subsp. *thermophilus*	[2–5]
Gastroenteritis		
Acute diarrhea	LAB, *Bifidobacterium* species, or *Saccharomyces boulardii*	[6–17]
Antibiotic-associated diarrhea	LAB or *S. boulardii*	[18–24]
Traveler's diarrhea	LAB	[25, 26]
Allergies	LAB	[27–31]
Clostridium difficile–induced colitis	LAB	[32–34]
Dental caries	LAB	[35]
Intestinal inflammation in children with cystic fibrosis	LAB	[36]
Respiratory infection in children	LAB	[37]
Nasal colonization with pathogens	LAB	[38]
Inflammatory bowel disease or irritable bowel syndrome	LAB and *Bifidobacterium* species, *S. boulardii* and drug, *S. boulardii* alone, or LAB alone	[39–43]

NOTE. LAB, lactic acid bacteria.

studies were done with infants or children, the etiologic agent was either rotavirus or unknown, and the probiotic used was *Lactobacillus rhamnosus* strain GG (*Lactobacillus* GG) (ATCC 53103) [7–14]. Other probiotics that have shown positive results for the treatment of acute gastroenteritis include *Lactobacillus reuteri* and *Saccharomyces boulardii* [15–17]. The European Society for Pediatric Gastroenterology, Hepatology, and Nutrition conducted the most extensive trial using *Lactobacillus* GG for the treatment of moderate-to-severe diarrhea in children [7]. The study included 287 children aged 1–36 months from 10 countries. The patients were randomized to be given either placebo or *Lactobacillus* GG along with the standard treatment, oral rehydration solution. Patients who received *Lactobacillus* GG had decreased severity and shorter duration of illness and a shorter hospital stay and were found to have a decreased likelihood of persistent diarrheal illness [7]. A similar study was conducted with 137 children aged 1–36 months who were admitted to the hospital with diarrhea and were randomized to receive placebo or *Lactobacillus* GG plus oral rehydration solution. Children given *Lactobacillus* GG had a significantly shorter duration of illness [8]. A study of 26 children in Thailand with watery diarrhea showed a significantly shorter duration of symptoms for those who received treatment with *Lactobacillus* GG [9]. A similar investigation involving 40 children that was conducted in Pakistan found that those who received treatment with *Lactobacillus* GG were less likely to have persistent diarrhea and had fewer episodes of vomiting, compared with the placebo group [10]. In a preventive study of 81 children aged 1–36 months who were hospitalized for illnesses other than diarrhea, symptoms of hospital-acquired rotavirus gastroenteritis were prevented by administration of *Lactobacillus* GG [12]. In another prevention study conducted in Peru, 204 children aged 6–24 months who were undernourished were randomized to receive placebo or *Lactobacillus* GG. There was a significant decrease in the rate of incidence of diarrhea among the children who received *Lactobacillus* GG who were not being breast-fed [14]. In one study, *Lactobacillus reuteri* was shown to shorten the duration of diarrhea in children [15]. In a clinical trial involving 130 children, *S. boulardii* was found to be effective for the treatment of acute diarrhea in children [16], and, in another study of 92 adults, a similar finding was reported [17].

Antibiotic-associated diarrhea. There have been a number of studies of the ability of probiotics to reduce the frequently observed intestinal adverse effects and diarrhea associated with the clinical use of antibiotics [18–24]. In a study of 119 children who received antibiotics for respiratory infections, during the first 2 weeks after antibiotic treatment began, the group receiving *Lactobacillus* GG had an ~70% reduction in diarrheal symptoms, compared with the group receiving placebo [18]. In another study, in which 202 children receiving oral antibiotics were followed, 8% of the children who received *Lactobacillus* GG concurrently with antibiotics experienced diarrheal symptoms, compared with 26% of the placebo group [19]. In 2 studies involving 60 and 120 adult patients who received antibiotic treatment to eliminate *Helicobacter pylori,* a significantly lower number of patients experienced nausea and diarrhea when they simultaneously received *Lactobacillus* GG versus placebo [20, 22]. There have been a number of studies that used other bacterial probiotics to treat antibiotic-associated diarrhea in which the treatment was not successful [24]. There are, however, at least 3 published studies that demonstrate the ability of *S. boulardii* to reduce antibiotic-associated diarrhea [24].

Traveler's diarrhea. People traveling to warmer climates and less-developed countries experience a high incidence of

diarrhea, often in the 50% range. A published study that tracked Finnish travelers to Turkey found that, at 1 of 2 resorts, oral ingestion of *Lactobacillus* GG conferred a significant protection rate, of 39.5% and 27.9%, in weeks 1 and 2 of the study, respectively. In the other resort area, no protection from consumption of *Lactobacillus* GG was noted [25]. A possible explanation for the discrepancy between the 2 resort sites is the availability of adequate refrigeration facilities, which is particularly relevant for probiotic preparations in warm climate situations. Also studied were 245 travelers from New York who went to various developing countries for periods of 1–3 weeks [26]. The travelers were provided *Lactobacillus* GG or a placebo, and *Lactobacillus* GG afforded a protection rate of 47%.

Prevention and treatment of allergic reactions. The most extensive studies of the modification of allergic reactions have been reported for atopic eczema with *Lactobacillus* GG as the probiotic [27–31]. There has also been a study that reported the use of *Bifidobacterium animalis* Bb12 to reduce the severity of atopic dermatitis [30]. In one study, 159 pregnant women with a family history of atopic disease were given either *Lactobacillus* GG capsules or a placebo for 2–4 weeks before their expected delivery date [27]. Mothers who chose to breast-feed their newborns continued to receive *Lactobacillus* GG or placebo for 6 months, and women who did not breast-feed gave the *Lactobacillus* GG or placebo to their infants. There was a 50% reduction in the frequency of atopic eczema in the first 2 years of the children's lives for the group given *Lactobacillus* GG. The breast milk of the mothers in the *Lactobacillus* GG group had higher levels of transforming growth factor β_2. In a follow-up study [28], the group that received *Lactobacillus* GG still had a significantly lower percentage of atopic eczema 4 years after birth, compared with the placebo group. In another study, 27 infants with atopic eczema were randomized into 3 groups, given *Lactobacillus* GG, *Bifidobacterium lactis* Bb12, or placebo. After 2 months, the SCORAD score, reflecting the extent and severity of atopic eczema, indicated a significant improvement in the skin condition of patients given probiotic-supplemented formulas ($P = .002$) [30]. A similar study involving 31 infants with atopic eczema who were removed from exposure to cow milk and were given either *Lactobacillus* GG or a placebo showed that treatment with *Lactobacillus* GG resulted in a significant improvement in their conditions that was not observed in the placebo group [31].

Treatment of relapsing gastoenteritis induced by* Clostridium difficile *toxin. Secondary to antibiotic treatment, disturbance of the intestinal flora can result in *C. difficile* growth and toxin production in the intestinal tract [32]. There have been several studies that showed that treatment with *Lactobacillus* GG prevents relapse of gastroenteritis after use of antibiotics. Clinical experience has shown a 60% relapse rate after therapy with metronidozole or vancomycin. Only 16% of patients who received *Lactobacillus* GG experienced a relapse, and, after a second course of *Lactobacillus* GG, there was a 94% overall cure rate [33, 34].

Prevention of dental caries. Children in a day care center who were given *Lactobacillus* GG for 7 months were examined for dental caries, and the children in the 3–4-year-old age group had significantly lower rates of dental caries and a reduced oral count of *Streptococcus mutans* compared with before the treatment [35].

Elimination of nasal pathogens. In a study of 209 healthy subjects, the consumption of a fermented milk product containing probiotics resulted in a significantly higher proportion of subjects with pathogenic bacteria eliminated from the nasal cavity, compared with consumption of a yogurt drink in the placebo group [38]. The pathogens removed included *Staphylococcus aureus, Streptococcus pneumoniae,* and β-hemolytic streptococci.

Treatment and prevention of relapses of inflammatory bowel disease. One of the major potential applications of probiotics is for the treatment and prevention of relapses of Crohn disease, ulcerative colitis, and irritable bowel syndrome. There have been reports of beneficial effects for inflammatory bowel disease that resulted from the administration of *Lactobacillus salivarius* [39], *Escherichia coli* strain Nissle [40], *S. boulardii* [41], and VSL#3 (VSL Pharmaceuticals), a mixture of probiotics [42]. These studies found fewer relapses and reduced steroid use among patients who received these probiotics. However, the studies were small, and the results were equivocal. There has been a report that VSL#3 reduced symptoms in patients with irritable bowel syndrome [43].

POTENTIAL MEDICAL INDICATIONS FOR PROBIOTICS IN THE FUTURE

Several diseases and conditions have been proposed to be treatable with probiotics on the basis of animal studies, preliminary human studies, uncontrolled studies, anecdotal observations, or simply speculation. These uses can be classified as potential applications of probiotics in the future or that require ongoing research. There have been animal studies and one small human trial that indicate that *Lactobacillus* GG may be useful for alleviating joint symptoms among patients with rheumatoid arthritis [44, 45]. There are several animal studies that show that probiotics inhibit initiation or progression of colon and bladder cancers [46, 47]. In vitro, cell culture, and animal studies have indicated that probiotics bind and prevent the absorption of aflotoxins, which have been implicated in the etiology of liver cancer in humans [48, 49]. A rat model of ethanol-induced liver damage has been used to demonstrate the protective effects of probiotics [50]. An animal model of diabetes showed that *Lactobacillus* GG could lower levels of blood hemoglobin A1c and could improve glucose tolerance [51]. Probiotics studied

Table 2. Present and future clinical applications of probiotics, by level of evidence of efficacy.

Applications with strong evidence
Gastroenteritis
Acute
Antibiotic associated
Applications with substantial evidence of efficacy
Allergic reactions, specifically atopic dermatitis
Applications that have shown promise
Childhood respiratory infection
Dental caries
Nasal pathogens
Relapsing *Clostridium dificile*–induced gastroenteritis (prevention)
Inflammatory bowel disease
Potential future applications
Rheumatoid arthritis
Irritable bowel syndrome
Cancer (prevention)
Ethanol-induced liver disease
Diabetes
Graft-versus-host disease

in a mouse model have demonstrated a possible role for these agents in the prevention or treatment of graft-versus-host disease in transplant recipients [52].

CONCLUSIONS

The current and proposed uses of probiotics cover a wide range of diseases and ailments. An attempt has been made to classify the quality of evidence that supports these various applications [53]. These classifications are based on existing studies, most of which are cited in this article, and not on an exhaustive review of the entire literature on probiotics. The broad classifications include (table 2) applications with proven benefits, applications with substantial evidence that require additional support, promising applications that need substantial additional evidence, and proposed future applications. Proven benefits of probiotics include the treatment of acute and antibiotic-associated diarrhea; applications with substantial evidence include the prevention of atopic eczema and traveler's diarrhea; promising applications include the prevention of respiratory infections in children, prevention of dental caries, elimination of nasal pathogen carriage, prevention of relapsing *C. difficile*–induced gastroenteritis, and treatment of inflammatory bowel disease; and proposed future applications include the treatment of rheumatoid arthritis, treatment of irritable bowel syndrome, cancer prevention, prevention of ethanol-induced liver disease, treatment of diabetes, and prevention or treatment of graft-versus-host disease. The use of probiotics in medical practice is rapidly increasing, as are studies that demonstrate the efficacy of probiotics. A note of caution should be applied: negative findings are being reported, as would be expected as more studies are being performed and as more applications are being sought for the use of probiotics. Overall, probiotics appear to be here to stay as part of the physician's armamentarium for the prevention and treatment of disease; however, more evidence-based research is required to firmly establish medical areas of use and areas in which probiotics are not applicable.

Acknowledgments

S.L.G., the journal's editor, was not involved in the editorial review or decision to publish this article.

Supplement sponsorship. This article was published as part of a supplement entitled "Developing Probiotics as Foods and Drugs: Scientific and Regulatory Challenges," sponsored by the Drug Information Association, the National Institutes of Health National Center for Complementary and Alternative Medicine (1R13AT003805-01 to Patricia L. Hibberd), the California Dairy Research Foundation, Chr. Hansen, the Dannon Company, General Mills, Institut Rosell, and Yakult International.

Potential conflicts of interest. B.R.G. and S.L.G. had a patent and license agreement for *Lactobacillus* GG. The patent expired in June 2006, and the license in January 2007. There are currently no conflicts of interest.

References

1. Salminen S, Gibson C, Bouley MC, et al. Gastrointestinal physiology and function: the role of prebiotics and probiotics. Br J Nutr **1998**; 80(Suppl 1):S147–71.
2. Savaiano DA, Abou EA, Smith DE, Levitt MD. Lactose malabsorption from yogurt, sweet acidophilus milk, and cultured milk in lactose-deficient individuals. Am J Clin Nutr **1984**; 40:1219–23.
3. deVrese M, Stegelmann A, Richter B, Fenseau S, Love C, Schrezenneir J. Probiotics compensation for lactose insufficiency. Am J Clin Nutr **2001**; 73:421S–9S.
4. Kim HS, Gilliland SE. *Lactobacillus acidophilus* as dietary adjunct for milk to aid lactose digestion in humans. J Dairy Sci **1983**; 66:959–66.
5. Kolars JC, Levitt MD, Aouj M, Savaino DA. Yogurt—an antidigesting source of lactose. N Engl J Med **1984**; 310:1–3.
6. Allen SJ, Okoko B, Martinez E, Gregorio G, Dans LF. Probiotics for treating infectious diarrhea. Cochrane Database Syst Rev **2003**; 2: CD003048.
7. Guandalini S, Pensabene L, Zikri MA, et al. *Lactobacillus* GG administered in oral rehydration solution to children with acute diarrhea: a multicenter European trial. J Pediatr Gastroenterol Nutr **2000**; 30: 54–60.
8. Shornikova AV, Isolauri E, Burkanova L, Lukovnikova S, Vesikari T. A trial in the Karelian Republic of oral rehydration and *Lactobacillus* GG for treatment of acute diarrhea. Acta Paediatr **1997**; 86:460–5.
9. Pant AR, Graham SM, Allen SJ, et al. *Lactobacillus* GG and acute diarrhea in young children in the tropics. J Trop Pediatr **1996**; 42:162–5.
10. Raza S, Graham SM, Allen SJ, Sultana S, Cuevas L, Hart CA. *Lactobacillus* GG promotes recovery from acute nonbloody diarrhea in Pakistan. Pediatr Infect Dis J **1995**; 14:107–11.
11. Sepp E, Tamm E, Torm S, Lutsar I, Mikelsaar M, Salminen S. Impact of a *Lactobacillus* probiotic on the faecal microflora in children with shigellosis. Microecol Ther **1995**; 23:74–80.
12. Szajewska H, Kotowska M, Murkowicz JZ, Armanska M, Mikolajczyk W. Efficacy of *Lactobacillus* GG in prevention of nosocomial diarrhea in infants. J Pediatr **2001**; 138:361–5.
13. Mastretta E, Longo P, Laccisaglia A, et al. Effect of *Lactobacillus* GG and breast-feeding in the prevention of rotavirus nosocomial infection. J Pediatr Gastroenterol Nutr **2002**; 35:527–31.
14. Oberhelman RA, Gilman RH, Sheen P, et al. A placebo-controlled trial of *Lactobacillus* GG to prevent diarrhea in undernourished Peruvian children. J Pediatr **1999**; 134:15–20.
15. Shornikova AV, Cosas I, Mykkanen H, Salo E, Vesikari T. Bactiotherapy

with *Lactobacillus reuteri* in rotavirus gastroenteritis. Pediatr Infect Dis J **1997**; 16:1103–7.
16. Cetina-Sauri G, Sierra Basto G. Evaluation therapeutique de *Saccharomyces boulardii* chez des enfants souffrant de diarrhee aigue. Ann Pediatr **1994**; 41:397–400.
17. Höchter W, Chase D, Hegenhoff G. *Saccharomyces boulardii* in treatment of acute adult diarrhoea: efficacy and tolerance of treatment. Münch Med Wochen **1990**; 132:188–92.
18. Arvola T, Laiho K, Torkkeli S, et al. Prophylactic *Lactobacillus* GG reduces antibiotic-associated diarrhea in children with respiratory infections: a randomized study. Pediatrics **1999**; 104:e64.
19. Vanderhoof JA, Whitney DB, Antonson DL, Hanner TL, Lupo JV, Young RJ. *Lactobacillus* GG in the prevention of antibiotic-associated diarrhea in children. J Pediatr **1999**; 135:564–8.
20. Armuzzi A, Cremonini F, Ojetti V, et al. Effect of *Lactobacillus* GG supplementation on antibiotic-associated gastrointestinal side effects during *Helicobacter pylori* eradication therapy: a pilot study. Digestion **2001**; 63:1–7.
21. Cremonini F, Di Caro S, Covino M, et al. Effect of different probiotic preparations on anti-*Helicobacter pylori* therapy-related side effects: a parallel group, triple blind, placebo-controlled study. Am J Gastroenterol **2002**; 97:2744–9.
22. Armuzzi A, Cremonini F, Bartolozzi F, et al. The effect of oral administration of *Lactobacillus* GG on antibiotic-associated gastrointestinal side effects during *Helicobacter pylori* eradication therapy. Aliment Pharmacol Ther **2001**; 15:163–9.
23. Siitonen S, Vapaatalo H, Salminen S, et al. Effect of *Lactobacillus* GG yoghurt in prevention of antibiotic associated diarrhea. Ann Med **1990**; 22:57–9.
24. Marchand J, Vandenplas Y. Microorganisms administered in the benefit of the host: myths and facts. Eur J Gastroenterol Hepatol **2000**; 12: 1077–88.
25. Oksanen PJ, Salminen S, Saxelin M, et al. Prevention of travellers' diarrhea by *Lactobacillus* GG. Ann Med **1990**; 22:53–6.
26. Hilton E, Kolakowski P, Singer C, Smith M. Efficacy of *Lactobacillus* GG as a diarrheal preventive in travelers. J Travel Med **1997**; 4:41–3.
27. Kalliomaki M, Salminen S, Arvilommi H, Kero P, Koskinen P, Isolauri E. Probiotics in primary prevention of atopic disease: a randomized placebo-controlled trial. Lancet **2001**; 357:1076–9.
28. Rautava S, Kalliomaki M, Isolauri E. Probiotics during pregnancy and breast-feeding might confer immunomodulatory protection against atopic disease in the infant. J Allergy Clin Immunol **2002**; 109:119–21.
29. Kalliomaki M, Salminen S, Poussa T, Arvilommi H, Isolauri E. Probiotics and prevention of atopic disease: 4-year follow-up of a randomized placebo-controlled trial. Lancet **2003**; 361:1869–71.
30. Isolauri E, Arvola T, Sutas Y, Moilanen E, Salminen S. Probiotics in the management of atopic eczema. Clin Exp Allergy **2000**; 30:1604–10.
31. Majamaa H, Isolauri E. Probiotics: a novel approach in the management of food allergy. J Allergy Clin Immunol **1997**; 99:179–85.
32. Biller JA, Katz AJ, Flores AF, Buie TM, Gorbach SL. Treatment of recurrent *Clostridium difficile* colitis with *Lactobacillus* GG. J Pediatr Gastroenterol Nutr **1995**; 21:224–6.
33. Gorbach SL, Chang TW, Goldin BR. Successful treatment of relapsing *Clostridium difficile* colitis with *Lactobacillus* GG. Lancet **1987**; 2:1519.
34. Bennett RG, Gorbach SL, Goldin BR, et al. Treatment of relapsing *Clostridium difficile* diarrhea with *Lactobacillus* GG. Nutr Today **1996**; 31(Suppl):35S–8S.
35. Nase L, Hatakka K, Savilahti E, et al. Effect of long-term consumption of a probiotic bacterium, *Lactobacillus rhamnosus* GG, in milk on dental caries and caries risk in children. Caries Res **2001**; 35:412–20.
36. Bruzzese E, Raia V, Gaudiello G, et al. Intestinal inflammation is a frequent feature of cystic fibrosis and is reduced by probiotic administration. Aliment Pharmacol Ther **2004**; 20:813–9.
37. Hatakka K, Savilahti E, Ponka A, et al. Effect of long term consumption of probiotic milk on infections in children attending day care centers: double blind, randomized trial. Br Med J **2001**; 322:1327.
38. Gluck U, Gebbers JO. Ingested probiotics reduce nasal colonization with pathogenic bacteria (*Staphyloccus aureus, Streptococcus pneumoniae,* and β-hemolytic streptococci). Am J Clin Nutr **2003**; 77:517–20.
39. Mattila-Sandholm T, Blum S, Collins JK, et al. Probiotics: towards demonstrating efficacy. Trends Food Sci Technol **1999**; 10:393–9.
40. Malchow HA. Crohn's disease and *Escherichia coli:* a new approach in therapy to maintain remission of colonic Crohn's disease? J Clin Gastroenterol **1997**; 25:653–8.
41. Guslandi M, Mezzi G, Sorghi M, Testoni PA. *Saccharomyces boulardii* in maintenance treatment of Crohn's disease. Dig Dis Sci **2000**; 45: 1462–4.
42. Venturi A, Gionchetti P, Rizzello F, et al. Impact on the composition of the faecal flora by a new probiotic preparation: preliminary data on maintenance treatment of patients with ulcerative colitis. Aliment Pharmacol Ther **1999**; 13:1103–8.
43. Brigdi P, Vitali B, Swennen E, Bazzocchi G, Matteuzzi D. Effects of probiotic administration upon the composition and enzymatic activity of human fecal microbiota in patients with irritable bowel syndrome or funcational diarrhea. Res Microbiol **2001**; 152:735–41.
44. Baharav E, Mor F, Halpern M, Weinberger A. *Lactobacillus* GG bacteria ameliorate arthritis in Lewis rats. J Nutr **2004**; 134:1964–9.
45. Hatakka K, Martio J, Korpela M, et al. Effects of probiotic therapy on the activity and activation of mild rheumatoid arthritis—a pilot study. Scand J Rheumatol **2003**; 32:211–5.
46. Goldin BR, Gualtieri LJ, Moore RP. The effect of *Lactobacillus* GG on the initiation and promotion of DMH-induced intestinal tumors in the rate. Nutr Cancer **1996**; 25:197–204.
47. Lim BK, Mahendran R, Lee YK, Bay BH. Chemopreventive effect of *Lactobacillus rhamnosus* on growth of a subcutaneously implanted bladder cancer cell line in the mouse. Jpn J Cancer Res **2002**; 93:36–41.
48. Lahtinen SJ, Haskard CA, Ouwehand AC, Salminen SJ, Ahokas JT. Binding of aflatoxin B_1 to cell wall components of *Lactobacillus rhamnosus* strain GG. Food Addit Contam **2004**; 21:158–64.
49. Haskard C, Binnion C, Ahokas J. Factors affecting the sequestration of aflatoxin by *Lactobacillus rhamnosus* strain GG. Chem Biol Interact **2000**; 128:39–49.
50. Nanji AA, Khettry U, Sadrzadeh SM. Lactobacillus feeding reduces endotoxemia and severity of experimental alcoholic liver (disease). Proc Soc Exp Biol Med **1994**; 205:243–7.
51. Tabuchi M, Ozaki M, Tamura A, et al. Antidiabetic effect of *Lactobacillus* GG in streptozotocin-induced diabetic rats. Biosci Biotechnol Biochem **2003**; 67:1421–4.
52. Gerbitz A, Schultz M, Wilke A, et al. Probiotic effects on experimental graft-versus-host disease: let them eat yogurt. Blood **2004**; 103:4365–7.
53. Doron S, Snydman DR, Gorbach SL. *Lactobacillus* GG: bacteriology and clinical applications. Gastroenterol Clin North Am **2005**; 34: 483–98.

Table 2. CD Markers in Patients With AEDS Before and After Topical Tacrolimus Treatment

	Percentage, mean (SD)		*P* value[a]
	At baseline (n = 21 patients)	12 wk later (n = 21 patients)	
CD3	66.12 (9.73)	63.94 (7.71)	.42
$CD4^+$	37.87 (9.30)	37.44 (4.41)	.85
$CD8^+$	24.38 (6.85)	23.62 (4.10)	.68
HLA-DR IN CD3	12.69 (11.79)	10.25 (3.53)	.37
CD57 in CD8	14.75 (10.72)	13.8 (8.70)	.76
CD19	19.68 (8.19)	20.44 (7.29)	.73
CD5 in CD20	51.71 (9.96)	48.87 (10.70)	.36
CD16	10.44 (8.32)	11 (5.83)	.78
$CD3\text{-}CD56^+$	10.50 (7.97)	10.56 (5.85)	.99
CD45Ra in CD4	63.19 (15.51)	65.25 (8.1)	.58
CD25	12.37 (9.32)	10.20 (2.43)	.32
CD4/CD8	1.67 (0.69)	1.56 (0.30)	.80

Abbreviation: AEDS, atopic eczema dermatitis syndrome.
[a] Using the *t* test for paired data.

parently not associated with changes in tested T-lymphocyte markers and that the atopic condition did not influence the effect of tacrolimus ointment therapy in children with AEDS.

Furthermore, the lack of changes in T-lymphocyte subsets confirmed that topical tacrolimus therapy did not affect the immune system, in accordance with literature data reporting very low systemic absorption of tacrolimus, 0.03%, ointment, with blood concentrations of the drug ranging from 1 to 2.28 ng/mL.[8] Finally we suggest that monitoring of peripheral blood T-lymphocyte subsets during topical tacrolimus therapy did not add useful information in the evaluation of therapeutic success in children with AEDS.

SALVATORE LEONARDI, MD
GIOVANNA VITALITI, MD
LUCIA SPICUZZA, MD
MARIO LA ROSA, MD

Pediatric Department
University of Catania
Catania, Italy
leonardi@unict.it

1. Johansson SG, Bieber T, Dahl R, et al. Revised nomenclature for allergy for global use: report of the Nomenclature Review Committee of the World Allergy Organization, October 2003. *J Allergy Clin Immunol*. 2004;113:832–836.
2. Sato A, Tsuji K, Yamamura M, et al. Increased type cytokine expression by both CD4+ CD45RO+ T cells and CD8+ CD45RO+ T cells in blood circulation is associated with high serum IgE but not with atopic dermatitis. *J Invest Dermatol*. 1998;111:1079–1084.
3. Stiehm ER, Roberts RL, Kaplan MS, et al. Pneumococcal seroconversation after vaccination for children with atopic dermatitis treated with tacrolimus ointment. *J Am Acad Dermatol.* 2005;53(supp 2): S206–S213.
4. Boguniewicz M, Eichenfield LF, Hultsch T. Current management of atopic dermatitis and interruption of the atopic march. *J Allergy Clin Immunol*. 2003;112:S140–S150.
5. Kagi MK, Wuthrich B, Montano E, et al. Differential cytokine profiles in peripheral blood supernatants and skin biopsies from patients with different forms of atopic dermatitis, psoriasis and normal individuals. *Int Arch Allergy Immunol*. 1994;103:332–340.
6. Walker C, Kagi MK, Ingold P, et al. Atopic dermatitis: correlation of peripheral blood T cell activation, eosinophilia and serum factors with clinical severity. *Clin Exp Allergy*. 1993;23:145–153.
7. Leonardi S, Rotolo N, Vitaliti G, Spicuzza L, La Rosa M. IgE values and T-lymphocyte subsets in children with atopic eczema/dermatitis syndrome. *Allergy Asthma Proc*. 2007;28:529–534.
8. Krueger GG, Eichenfeld L, Goodman JJ, et al. Pharmacokinetics of tacrolimus following topical application of tacrolimus ointment in adult and pediatric patients with moderate to severe atopic dermatitis. *J Drugs Dermatol*. 2007;6:185–193.

PROBIOTIC GASTROINTESTINAL ALLERGIC REACTION CAUSED BY *SACCHAROMYCES BOULARDII*

Saccharomyces boulardii medication is a nonpathogenic yeast probiotic that is widely prescribed in a lyophilized form (so-called *S boulardii* lyo) in many countries. Modulation of commensal bacteria of the gut with probiotics has been shown to have an effect on the prevention of food allergy.[1] To our knowledge, there is no contraindication for the patients except for a rare case of fungemia.[2]

Several fungal species are known to cause cutaneous and respiratory allergic disease.[3] The ingestion of yeast can cause clustered sensitivity to fungi, including an anaphylactic reaction.[4] *Saccharomyces boulardii* is a subtype of *Saccharomyces cerevisiae* known as brewer's or baker's yeast. *Saccharomyces cerevisiae* causes baker's asthma.[5] However, to our knowledge, allergy caused by the ingestion of *S boulardii* lyo has not been reported. To our knowledge, we report the first case of a gastrointestinal allergic reaction that developed after the administration of *S boulardii* probiotic in a patient with food protein–induced enterocolitis syndrome (FPIES).

A 5-month-old exclusively breastfed boy was admitted to the hospital with vomiting, cyanosis, and lethargy within 4 hours after ingesting approximately 30 mL of soy formula. Two weeks later, an open standard oral food challenge (OFC) with soy formula was performed and soy intolerance was observed. After soy-induced FPIES was diagnosed on the basis of the diagnostic guidelines,[6,7] iron supplements and breastfeeding exclusively were continued, with no solid intake recommended. However, the mother gave the infant some fruits and vegetables even though we warned of FPIES reactions. Fortunately, no adverse reactions occurred.

At the age of 8 months, presumed bacterial colitis was treated with cefixime (Suprax; Dong-A Pharmaceutical Co, Seoul, Korea); *S boulardii* lyo as Bioflor (Kuhnil Pharmaceutical Co, Seoul, Korea, manufactured under the license of Biocodex, Gentilly, France) was also provided. Two hours later, after taking the medicines, the infant presented to the emergency department with repetitive vomiting and cyanosis and required intravenous fluid resuscitation. Cefixime was excluded as a cause for the infant's symptoms because subsequent exposure to the antibiotic was well tolerated. We presumed that an allergic reaction consistent with FPIES[6,7] was caused by Bioflor.

One week later, after the infant's bacterial colitis had resolved, an OFC with 1 pack of powdered Bioflor was conducted as described by previous suggestions.[6,7] After 2 hours, projectile vomiting was detected and soon lethargy with cyanosis followed. The infant's blood pressure was 74/39 mm Hg, and isotonic sodium chloride solution (30 mL/kg) was infused for 1 hour, followed by dextrose saline. After 5 hours of the challenge, the infant's blood pressure normalized to 92/65 mm Hg. No fever developed. In addition, there were no mucocutaneous or respiratory symptoms observed. Eight hours later, stools that were bloody and purulent and had a foul odor

Disclosures: Authors have nothing to disclose.

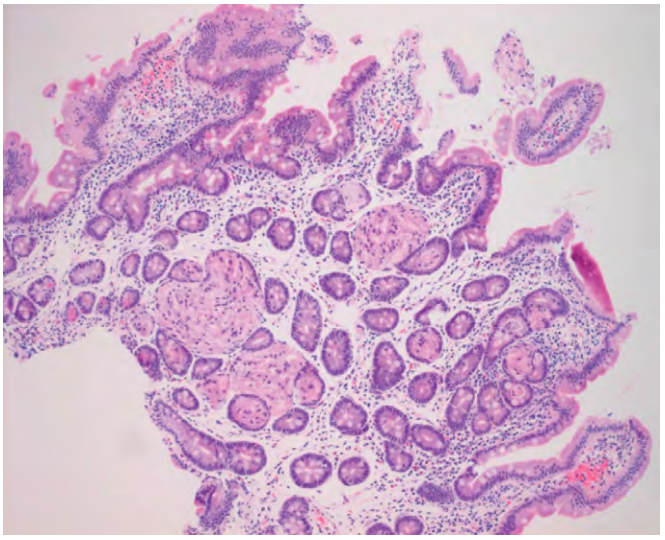


Figure 1. In a duodenal mucosal biopsy specimen, there is patchy villous atrophy and diffuse nonspecific inflammation with eosinophils and lymphoplasma cells, but no yeast is identified (hematoxylin-eosin, original magnification ×100).

were observed. The stool smear test showed many red and white blood cells. Both blood and stool culture results were negative. Multiple radioallergosorbent test (RIDA Allergy Screen Test; R-Biopharm AG, Darmstadt, Germany) results for yeast and fungi (not *Saccharomyces* or other related species) were all negative, and the serum IgE level was less than 17.6 IU/mL (reference range, 0–230 IU/mL). Neither prick skin testing nor patch testing to Bioflor was performed. The clinical findings were consistent with the diagnostic criteria[6,7] for an *S boulardii* lyo–induced gastrointestinal allergy, such as the FPIES adverse reaction. Neither the infant nor his mother had previously taken Bioflor or any other probiotics. A biopsy specimen of the duodenal mucosa, performed 24 hours after the FPIES-like reaction, showed patchy villous atrophy and diffuse nonspecific inflammation with eosinophils (20 cells per high-power field) and lymphoplasma cells, but no yeast was identified (Fig 1).

FPIES, manifesting as a sepsislike symptom, is the most severe form of non–IgE-mediated gastrointestinal food hypersensitivity in infants. The OFC test remains the diagnostic standard for this disorder. The food specific IgE tests are not helpful.[6,7] On the basis of the OFC and clinical diagnostic criteria, the present case demonstrated an FPIES-like allergic reaction caused by *S boulardii*.

Although FPIES is known to be caused by cow's milk and various solid foods, including rice,[8] there has been no report on cases caused by extracts of yeast. One powder pack of Bioflor is 765 mg and is mainly composed of 471.9 mg of fructose and 282.5 mg of *S boulardii* in a lyophilized form. Because this patient had no specific symptoms after ingestion of fruits, the fructose contained in the Bioflor was not influential in developing the FPIES-like allergic reaction. Bloody, purulent, and foul-smelling stools indicate that massive inflammation probably occurs in the bowel mucosa in the patient with FPIES undergoing an OFC.[7]

Although *S boulardii* lyo is a relatively safe probiotic, unexpected allergic reactions can develop. *Saccaromyces boulardii* probiotic should be cautiously administered in patients with latent allergies.

Jin-Bok Hwang, MD*
Kyung Ji Kang, MD*
Yu Na Kang, MD†
Ae Suk Kim, MD‡

*Department of Pediatrics
Dongsan Medical Center
Keimyung University School of Medicine
Daegu, Korea
†Department of Pathology
Dongsan Medical Center
Keimyung University School of Medicine
Daegu, Korea
‡Department of Pediatrics
Dongguk University College of Medicine
Gyeongju, Korea
pedgi@kmu.ac.kr

1. Savilahti E, Kuitunen M, Vaarala O. Pre and probiotics in the prevention and treatment of food allergy. *Curr Opin Allergy Clin Immunol*. 2008;8:243–248.
2. Enache-Angoulvant A, Hennequin C. Invasive *Saccharomyces* infection: a comprehensive review. *Clin Infect Dis*. 2005;41:1559–1568.
3. Mari A, Schneider P, Wally V, et al. Sensitization to fungi: epidemiology, comparative skin tests, and IgE reactivity of fungal extracts. *Clin Exp Allergy*. 2003; 33:1429–1438.
4. Airola K, Petman L, Makinen-Kiljunen S. Clustered sensitivity to fungi: anaphylactic reactions caused by ingestive allergy to yeasts. *Ann Allergy Asthma Immunol*. 2006;97:294–297.
5. Belchi-Hernandez J, Mora-Gonzalez A, Iniesta-Perez J. Baker's asthma caused by *Saccharomyces cerevisiae* in dry powder form. *J Allergy Clin Immunol*. 1996;97: 131–134.
6. Sicherer SH, Eigenmann PA, Sampson HA. Clinical features of food protein-induced enterocolitis syndrome. *J Pediatr*. 1998;133:214–219.
7. Hwang JB, Song JY, Kang YN, et al. The significance of gastric juice analysis for a positive challenge by a standard oral challenge test in typical cow's milk protein-induced enterocolitis. *J Korean Med Sci*. 2008;23:251–255.
8. Hojsak I, Kljaic-Turkalj M, Misak Z, et al. Rice protein-induced enterocolitis syndrome. *Clin Nutr*. 2006;25:533–536.

ANAPHYLACTIC REACTION TO PERMANENT TATTOO INK

Tattoos are increasingly prevalent in Western society. Delayed-hypersensitivity reactions to tattoo ink are well described in the literature, but, to our knowledge, anaphylaxis after permanent tattoos has never been reported.

A 30-year-old woman with suspected tattoo allergy was referred to our clinic for evaluation. Her medical history included seasonal allergic rhinitis, asthma, eczema, and animal allergies to dogs, cats, and horses. The patient received her first multicolored tattoo in 1993, with no adverse reactions. In June 1999, she received a black ink tattoo on her back without adverse reactions. One month later, she had colored ink added to the tattoo. Approximately 12 hours after the procedure, a feeling of heat began at her anus and spread rapidly over her body, followed by the development of hives over her face, chest, and upper back. She experienced acute shortness of breath and intractable abdominal pain. She went to the emergency department, where her symptoms resolved after she was treated with intravenous (IV) diphenhydramine, 2 doses of oral lorazepam, and IV droperidol. Throughout this episode, the tattoo appeared normal. Six months later, the patient returned for further coloring of her tattoo, at which time she experienced similar symptoms, occurring faster and resolving after a regimen of antihistamines and IV methylprednisolone sodium succinate (Solu-Medrol).

Disclosures: Authors have nothing to disclose.

Case Report

Saccharomyces cerevisiae oesophagitis in a patient with oesophageal carcinoma

Dimas Alexandre Kliemann[1], Vicente Sperb Antonello[2], Luis Carlos Severo[1,3,4], Alessandro Comarú Pasqualotto[1,5]

[1]*Pulmonary Sciences, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil*
[2]*Infection Control Department, Hospital Fêmina, Porto Alegre, Brazil*
[3]*Mycology Laboratory, Complexo Hospitalar Santa Casa de Porto Alegre, Porto Alegre, Brazil*
[4]*Department of Internal Medicine, Universidade Federal do Rio Grande do Sul, Brazil. Researcher for the CNPq (level 1B)*
[5]*Molecular Biology Laboratory, Complexo Hospitalar Santa Casa de Porto Alegre, Porto Alegre, Brazil*

Abstract

Saccharomyces species are emerging opportunistic fungal pathogens that can cause bloodstream infections in humans. These infections have often been associated with the ingestion of probiotics. *Saccharomyces* oesophagitis is a rare condition which has been described so far in only two publications. Here we report the case of a patient who was diagnosed with *Saccharomyces* oesophagitis. The clinical picture was indistinguishable from that of *Candida* oesophagitis. The *Saccharomyces* isolate was shown to be susceptible to fluconazole by both CLSI M27-A and disk diffusion methods. In contrast to cases of fungaemia, *Saccharomyces* oesophagitis does not seem to follow probiotic use. Due to the potential for antifungal resistance among emerging fungal pathogens, proper mycological identification at the species level is essential.

Key words: opportunistic infections; oesophagitis; saccharomyces; yeasts; probiotics; cancer.

J Infect Dev Ctries 2011; 5(6):493-495.

(Received 04 October 2010 – Accepted 31 December 2010)

Introduction

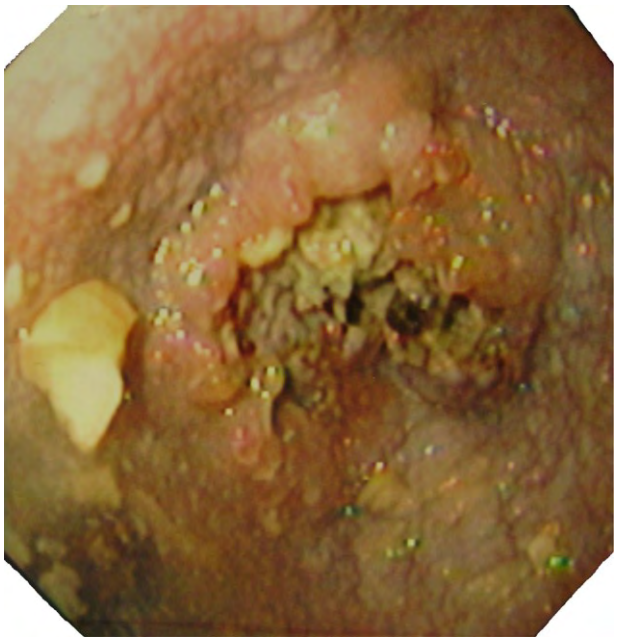
Saccharomyces spp. are well-known ubiquitous ascomycetous yeasts used in food business, including baking and brewing industries. Additionally, *Saccharomyces* spp. have been used as nutritional supplements and administered to treat antibiotic-associated diarrhoea [1]. Although these fungi are colonisers of human mucosae [2], a higher number of reports have linked *Saccharomyces cerevisiae* to human infections as an opportunistic agent.

Fungaemia is the most frequent manifestation of *Saccharomyces* infection in humans, mostly associated with contaminated catheters. However, some cases of *Saccharomyces* fungaemia following the use of probiotics have been reported [3]. Other disease manifestations as *Saccharomyces* oesophagitis are rare. Here we describe a case of *Saccharomyces* oesophagitis affecting a patient with oesophageal carcinoma. We used the criteria proposed by Konecny *et al*. to differentiate colonisation from infection [4].

Case report

A 47-year-old man was admitted to the hospital complaining of a three-month history of progressive dysphagia and weight loss. He gave a history of retrosternal chest pain, nausea, poor appetite, and dyspnoea. During his hospital stay, a single febrile episode was documented (38ºC). Computerised tomography of the chest revealed a dilated oesophagus with a residual liquid content extending to beyond the epiphrenic segment. Thickening of the oesophageal wall was observed, with associated oesophageal obstruction. Oesophagogastroduodenoscopy revealed an exophytic lesion in the upper third of the oesophagus which blocked progression of the endoscope. The mucosa proximal to the vegetant lesion showed white plaques resembling *Candida* oesophagitis. A biopsy was performed, and histopathologic studies revealed the presence of a moderately differentiated epidermoid carcinoma of the oesophagus. In addition to acanthosis and focal areas of necrosis, hyaline

Figure 1. Hyperemia and the presence of fungal elements in the upper esophagus.



hyphae were also seen. Direct mycological examination showed presence of yeasts, and fungal culture on Sabouraud-Cloranfenicol at 25ºC revealed growth of *S. cerevisiae.* The fungus was identified at the species level using ID 32C system (bioMeriex, Marcy l'Etoile, France) [5]. Disk diffusion assay using the Clinical and Laboratory Standards Institute (CLSI) reference broth microdilution method M27-A produced a halo of > 30 mm, indicating susceptibility to fluconazole at MIC 0.125 µg/ml) [6]. A 7-day course of fluconazole 400 mg/daily resulted in remission of the clinical symptoms (*i.e.*, retrosternal chest pain, nausea, and dyspnoea). The patient was discharged from the hospital with a plan for further oesophagectomy but he died seven days after leaving the hospital. An autopsy was not performed.

Discussion

Saccharomyces oesophagitis is a rare disease, reported so far in only two publications. Eng *et al*. [7] reported in 1984 that a patient with Waldenstrom's macroglobulinaemia had a mixed oesophageal infection caused by *S. cerevisiae* and *Candida albicans*. The patient received no antifungal therapy and died two days after the diagnosis of causes other than the *Saccharomyces* infection. In another report [4], an HIV-infected patient with *Saccharomyces* oesophagitis was successfully treated with fluconazole. However, no details about sampling procedures and histopathological results were given. The isolate was found to be resistant to itraconazole and showed a high MIC for fluconazole (8 µg/ml). Similarly, in our patient, none of the previous reports associated *Saccharomyces* oesophagitis with the use of probiotics [4, 7]. Although there is no information about the use of probiotics in our case report because the data are from medical records, it is believed that the disease was caused by food ingestion.

S. cerevisiae has been increasingly implicated as a human opportunistic pathogen. This probably results not only from improvements in laboratory techniques allowing identification of fungi at the species level, but also from the growing clinical experience achieved in the last decades in the recognition of fungal infections. Moreover, humans are progressively more exposed to *S. cerevisiae* by oral ingestion, because it is believed to be of great nutritional value.

Although *S. cerevisiae* can colonise the oesophageal mucosa without causing infection, the patient described in this report presented all four criteria suggested by Konecny *et al*. to differentiate colonisation from infection [4]. These are (1) presence of clinical symptoms; (2) histopathological evidence for tissue invasion; (3) absence of other organisms recovered in culture; and (4) therapy based on susceptibility tests. In both previous cases of *S. cerevisiae* oesophagitis, these criteria were not

completely fulfilled [4, 7]; therefore, the differentiation between colonisation and infection was not possible.

Due mainly to the low frequency of *Saccharomyces* infections, the best therapeutic approach has not yet been defined. Whilst *S. cerevisiae* has been consistently susceptible to both amphotericin B (MICs of 0.5-1 µg/ml) and 5-flucytosine (0.25 µg/ml), different rates of resistance to fluconazole and itraconazole have been reported [8, 9]. Resistant strains of *Saccharomyces* species can emerge in units where fluconazole is extensively used [10]. Both posaconazole and voriconazole have been reported to have good *in vitro* activity against this fungus [9, 11]. Although the isolate recovered from our patient was susceptible *in vitro* to fluconazole, it should be noted that neither the CLSI nor the disk diffusion method have been formally approved for testing *S. cerevisiae* susceptibility.

In a recent review of the literature [3], 60 cases of *Saccharomyces* fungaemia were found. Interestingly, the majority of cases (54.6%) followed the use of probiotics by a median time of 10 days. It is also of interest that in this review only three patients were healthy before the bloodstream infection (two had self-inflicted fungaemia and one patient ingested large quantities of brewer's yeast as a nutritional supplement). Other clinical syndromes associated with *Saccharomyces* infection included endocarditis, aortic graft infection, tracheo-bronchitis, pneumonia, empyema, liver abscess, peritonitis, vaginitis, urinary tract infection, fever of unknown origin, and cellulites [3].

In conclusion, although *S. cerevisiae* is a rare aetiology of oesophagitis, this opportunistic organism should not be dismissed as non-pathogenic in the presence of the criteria suggested by Konecny *et al*. Since the clinical picture of *Saccharomyces* oesophagitis is indistinguishable from oesophagitis caused by other pathogens, a search for the aetiological agent seems worthy. The emergence of new fungal pathogens in cases of oesophagitis – particularly those with reduced susceptibility to azole anti-fungals – reinforces the importance of proper mycological examination of these samples.

Acknowledgments

We thank Dr Valério Aquino for providing susceptibility results.

References

1. Billoo AG, Memon MA, Khaskheli SA, Murtaza G, Iqbal K, Saeed Shekhani M, Siddiqi AQ. (2006) Role of a probiotic (*Saccharomyces boulardii*) in management and prevention of diarrhoea. World J Gastroenterol 12: 4557-4560.
2. Salonen JH, Richardson MD, Gallacher K, Issakainen J, Helenius H, Lehtonen OP, Nikoskelainen J. (2000) Fungal colonization of haematological patients receiving cytotoxic chemotherapy: emergence of azole-resistant *Saccharomyces cerevisiae*. J Hosp Infect 45: 293-301.
3. Munoz P, Bouza E, Cuenca-Estrella M, Eiros JM, Perez MJ, Sanchez-Somolinos M, Rincón C, Hortal J, Peláez T. (2005) *Saccharomyces cerevisiae* fungemia: an emerging infectious disease. Clin Infect Dis 40: 1625-1634.
4. Konecny P, Drummond FM, Tish KN, Tapsall JW (1999) *Saccharomyces cerevisiae* oesophagitis in an HIV-infected patient. Int J STD AIDS 10: 821-822.
5. BioMerieux (1993) API ID 32 C – Instruction manual. BioMerieux, Marcy l'Etoile, France.
6. CLSI (2002) Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts; Approved Standard - Second Edition. CLSI document M27-A2 (ISBN 1-56238-469-4). CLSI, 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898 USA.
7. Eng RH, Drehmel R, Smith SM, Goldstein EJ (1984) *Saccharomyces cerevisiae* infections in man. Sabouraudia 22: 403-407.
8. Richter SS, Galask RP, Messer SA, Hollis RJ, Diekema DJ, Pfaller MA (2005) Antifungal susceptibilities of *Candida* species causing vulvovaginitis and epidemiology of recurrent cases. J Clin Microbiol 43: 2155-2162.
9. Swinne D, Watelle M, Van der Flaes M, Nolard N (2004) *In vitro* activities of voriconazole (UK-109, 496), fluconazole, itraconazole and amphotericin B against 132 non-*albicans* bloodstream yeast isolates (CANARI study). Mycoses 47:177-183.
10. Tiballi RN, Spiegel JE, Zarins LT, Kauffman CA (1995) *Saccharomyces cerevisiae* infections and antifungal susceptibility studies by colorimetric and broth macrodilution methods. Diagn Microbiol Infect Dis 23: 135-140.
11. Pfaller MA, Messer S, Jones RN (1997) Activity of a new triazole, Sch 56592, compared with those of four other antifungal agents tested against clinical isolates of *Candida* spp. and *Saccharomyces cerevisiae*. Antimicrob Agents Chemother 41: 233-235.

Corresponding author

Vicente Sperb Antonello
Rua Mostardeiro, 17
Controle de Infecção, Hospital Fêmina
Porto Alegre, Brazil CEP: 91430-001
Telephone: +55 51 3314-5239
Fax: +55 51 33421330
Email: vicente_antonello@hotmail.com

Conflict of interest: No conflict of interests is declared.

Gut, 1992, 33, 909-913

Antibody (IgG, IgA, and IgM) to baker's yeast (*Saccharomyces cerevisiae*), yeast mannan, gliadin, ovalbumin and betalactoglobulin in monozygotic twins with inflammatory bowel disease

E Lindberg, K-E Magnusson, C Tysk, G Järnerot

Abstract

To assess whether dietary antigens play a role in inflammatory bowel disease, 26 monozygotic twin pairs with inflammatory bowel disease and 52 healthy controls were investigated for serum antibodies (IgA, IgG, IgM) against ovalbumin, betalactoglobulin, gliadin, whole yeast (*Saccharomyces cerevisiae*) and yeast cell wall mannan. The twins were made up of five pairs concordant and nine pairs discordant for Crohn's disease, and two pairs concordant and 10 pairs discordant for ulcerative colitis. Two patients with Crohn's disease had a slight increase in disease activity, the others were in clinical remission. Two striking observations were made: first, individuals with ulcerative colitis were indistinguishable from healthy twins, and controls except for the response to gliadin. Both healthy and diseased twins had higher IgA levels to gliadin than controls. Second, twins who had developed Crohn's disease displayed higher antibody titres towards yeast cell wall mannan in particular, but also to whole yeast (*Saccharomyces cerevisiae*) of all antibody types (IgA, IgG, and IgM). In contrast, the response to gliadin, ovalbumin, and betalactoglobulin did not differ from healthy twins and was even lower than in the controls. The results argue against an increased systemic antigen presentation caused by an impaired mucosal barrier in the inflammatory bowel disease. Rather, they suggest that yeast cell wall material - that is, mannan, or some antigen rich in mannose and cross reacting with mannan, may play an aetiological role in Crohn's disease, but not in ulcerative colitis. The increases in IgA and IgM, as well as IgG suggest that local and systemic immune systems are selectively activated by antigen(s) present in the cell wall of baker's yeast.

Departments of Medicine, Division of Gastroenterology and Pediatrics, Örebro Medical Center Hospital, Örebro and Medical Microbiology, University of Linköping, Linköping, Sweden
E Lindberg
K-E Magnusson
C Tysk
G Järnerot

Correspondence to:
Dr Eva Lindberg, Department of Pediatrics, Örebro Medical Center Hospital, S-701 85 Örebro, Sweden.

Accepted for publication
16 September 1991

Food antigens have been implicated in the aetiology of inflammatory bowel disease.[1-13] In particular cow's milk proteins have been thoroughly investigated.[1-10] The results differ possibly because of methodological differences.[11]

An increased occurence of gluten intolerance in patients with ulcerative colitis, has also been described.[14 15] Higher serum antibody titres to *Saccharomyces cerevisiae* (baker's yeast) were recently found in patients with Crohn's disease compared with patients with ulcerative colitis or healthy controls.[12] The response was specific for *Saccharomyces cerevisiae* strains, and did not include yeast such as *Candida albicans*.[13]

Antibody levels to a potent immunogenic egg protein, ovalbumin, do not appear to have been studied much in inflammatory bowel disease.[2 16]

The reason for putatively higher antibody titres to dietary antigens in inflammatory bowel disease might be a defect in the intestinal barrier allowing macromolecules to pass without being degraded.[17-19] Hollander *et al*[18 19] described an increased permeability not only in patients with Crohn's disease but also in their healthy relatives. Thus, the main pathogenetic factor in Crohn's disease might be permeability disorder, possibly genetically determined.

This study concerns the antibody response to various dietary antigens in patients with either Crohn's disease or ulcerative colitis, and their healthy monozygotic twins, aiming at delineating antigen specific reactions and genetic associated predispositions for inflammatory bowel disease.

The study was approved by the Local Ethical Committee, Örebro Medical Center Hospital, Örebro, Sweden.

Methods

SUBJECTS

By matching the Swedish twin registry at the Department of Environmental Hygiene, Karolinska Institute, Stockholm, with the central diagnosis register of hospital inpatients at the National Board of Health and Welfare, Stockholm, a population of monozygotic or dizygotic twins of the same sex has been identified and described earlier.[22] Thirty four monozygotic pairs with inflammatory bowel disease were found. Those who were younger than 75 years of age and with both twins in each pair still alive were invited to participate in the present investigation.

Two recently diagnosed monozygotic pairs with inflammatory bowel disease were also invited. Fifty two of 66 subjects agreed to participate constituting 14 pairs with Crohn's disease and 12 with ulcerative colitis. Five Crohn's disease twin pairs were concordant for the disease and two ulcerative colitis pairs.

The zygosity classification of the Swedish twin registry was used, which relies on questions about childhood resemblance. It has proved to

be very accurate, and in monozygotic twins a correct classification is obtained in 99% in comparison with serological methods.[23]

CONTROLS

One healthy control for each twin was chosen from members of the staff or blood donors, all without any history of gastrointestinal disease. In total, 52 persons were matched for sex and age within two years.

BLOOD SAMPLING

Venous blood was obtained and allowed to clot at room temperature and centrifuged before withdrawal of serum. Aliquots of serum were frozen at −70°C until analysed.

DETERMINATION OF ANTIBODIES TO FOOD ANTIGENS

Protein antigen preparations were obtained from Sigma Chemical Co (St Louis, Mo, USA) – namely, ovalbumin (A 5503), betalactoglobulin (L 0130), gliadin (G 3375), and mannan (M 3640). Whole yeast cell antigen was prepared in the following way. The cells of ordinary baker's yeast, *Saccharomyces cerevisiae*, were boiled for one hour, diluted to 25 μg/ml and usually used directly, or stored at −20°C until use.

To prepare antigen solutions, ovalbumin and betalactoglobulin were diluted in phosphate buffered saline, pH 7·3 to 25 μg/ml, and yeast cell wall mannan to 100 μg/ml, whereas gliadin was dissolved in 70% ethanol and diluted with phosphate buffered saline to 100 μg/ml. Whole yeast extract[12] was used at 25 μg/ml.

As antisera, horseradish-peroxidase conjugated rabbit antihuman immunoglobulins were used (Dakopatts, Stockholm, Sweden) – that is, anti-IgG (type P 214), anti-IgA (P 216), and anti-IgM (P 215). Two hundred microlitres antigen solution was added to each well of the microtitre-plate (96 well plate, Haegar Plastics, Oslo, Norway), incubated for two hours at 37°C, and incubated overnight in a moist chamber. The microtitre plates were then washed three times with 250 μl phosphate buffered saline with Tween 20 (0·05%).

Patients' sera were diluted 1:25 in phosphate buffered saline-Tween and heat inactivated in a water bath for 30 minutes at 56°C. Then, 200 μl of each serum sample was added to three separate wells for each antigen and incubated for one hour at 37°C; thereafter, the wells were washed three times with phosphate buffered saline-Tween and incubated with 200 μl horseradish-peroxidase conjugates (diluted 1:200 with phosphate buffered saline-Tween), washed again with phosphate buffered saline-Tween, and followed by substrate solution. The plates were light protected until they were read, and the reaction was stopped after 30 minutes by adding 50 μl 12·5% sulphuric acid. The substrate containing 100 ml phosphate buffered saline, 0·4 ml hydrogen peroxide (2%), and 10 ml 1,4-phenylenediamine dissolved in methanol (10 mg/ml) was freshly prepared and kept in the dark before use. Absorbance was read with an automatic ELISA reader (Immuno-Reader NJ-2000, Nippon Intermed KK, Japan).

To obtain a positive control in each plate, serum from a volunteer with measurable levels of anti ovalbumin, anti betalactoglobulin, and anti gliadin of all immunoglobulin classes was used. For negative controls, sera with negligible specific antibody activity to these antigens was used in addition to phosphate buffered saline-Tween alone. The reference positive control serum was obtained during the period of investigation, frozen at −20°C in aliquots suitable for one day of experiments. When a deviation >10% was noted between a positive or negative control measurement, and accumulated median of corresponding controls the whole determination was repeated. To further reduce the influence of methodological errors, the median value of the three samples was used for calculations, and all analyses were performed by the same technician with the same equipment throughout the study. The results were expressed in arbitrary absorbance units per millimetre (absorbance units/ml) without subtraction of background, which was ≤0·07, 0·06, and 0·06 absorbance units/ml for IgG, IgA, and IgM, respectively. The overall reproducibility over the whole measurement range was within 2% for triple samples.

STATISTICAL ANALYSIS

When comparing Crohn's disease and ulcerative colitis disease twins, healthy twins, and controls, Mann-Whitney U test and Student's *t* test were used. Wilcoxon's sign rank test was used when comparing pairs, where only one individual had developed the disease.

Results

PATIENT CHARACTERISTICS

Patients with Crohn's disease had a mean age at diagnosis of 28·5 years (range 20–45), and the actual mean age was 42·9 years (range 34–63). In patients with ulcerative colitis the mean age at diagnosis was 27·7 years (range 17–45), and the actual mean age was 49·1 years (range 24–74). Two patients with Crohn's disease (Crohn's disease twin) had mild diarrhoea and slightly increased serum C-reactive protein and orosomucoid levels, which was treated with sulphasalazine. The other patients were inactive and the only therapy was vitamins or loperamide (Imodium, Janssen Pharmaceutica, Beerse, Belgium). Two patients were on a lactose reduced diet, but only one had verified lactose intolerance. Eight patients were on a fat reduced diet. One of the two patients with increased disease activity was on tube feeding with elemental diet (Reabilan, Roussel Nordiska AB, Stockholm, Sweden) plus milk. All patients with ulcerative colitis (ulcerative colitis twin) were in clinical remission and had normal levels of haemoglobin, C-reactive protein and serum orosomucoid. Six were treated with sulphasalazine. One healthy twin was treated with prednisolone (5 mg on alternate days) for chronic hepatitis. None of the ulcerative colitis twins nor their healthy twins were on a special diet. A

thorough interview did not reveal symptoms suggesting inflammatory bowel disease in the healthy twins. They had remained healthy for an average 14·9 years (range 7–31) and 21·4 years (range 8–40) after diagnosis in the Crohn's disease and ulcerative colitis groups, respectively.

Sigmoidoscopy was performed in the subjects, except in five patients with ulcerative colitis and three patients with Crohn's disease who had had a proctocolectomy and in one Crohn's disease twin pair where both had severe perianal disease preventing sigmoidoscopy. Patients as well as healthy twins showed an inactive rectal mucosa on macroscopic and microscopic assessment.

ANTIBODY LEVEL

General

There was a considerable individual variation in antibody response, especially for IgG to all tested antigens but also the level of IgA and IgM to yeast cell wall mannan and whole yeast (*Saccharomyces cerevisiae*) varied much. For this reason a non-parametric method, Mann-Whitney U test, as well as a parametric method (Student's *t* test) were used to analyse the results. A few general observations can be made based on the material presented in the Figure and the Table. In ulcerative colitis twins the antibody response (IgA, IgG, IgM) to tested antigens were similar to that of the healthy twin of patient with ulcerative colitis twins and controls with a few exceptions. In contrast, Crohn's disease twins had higher titres of IgA, IgG, and IgM to yeast cell wall mannan and IgA to whole yeast (*Saccharomyces cerevisiae*), but not to the other dietary antigens.

Disease location did not have any influence on antibody response with one exception. Crohn's disease patients with small bowel disease only displayed higher IgG to whole yeast (*Saccharomyces cerevisiae*) than those with combined small and large bowel disease ($t=2·469$, $p=0·024$).

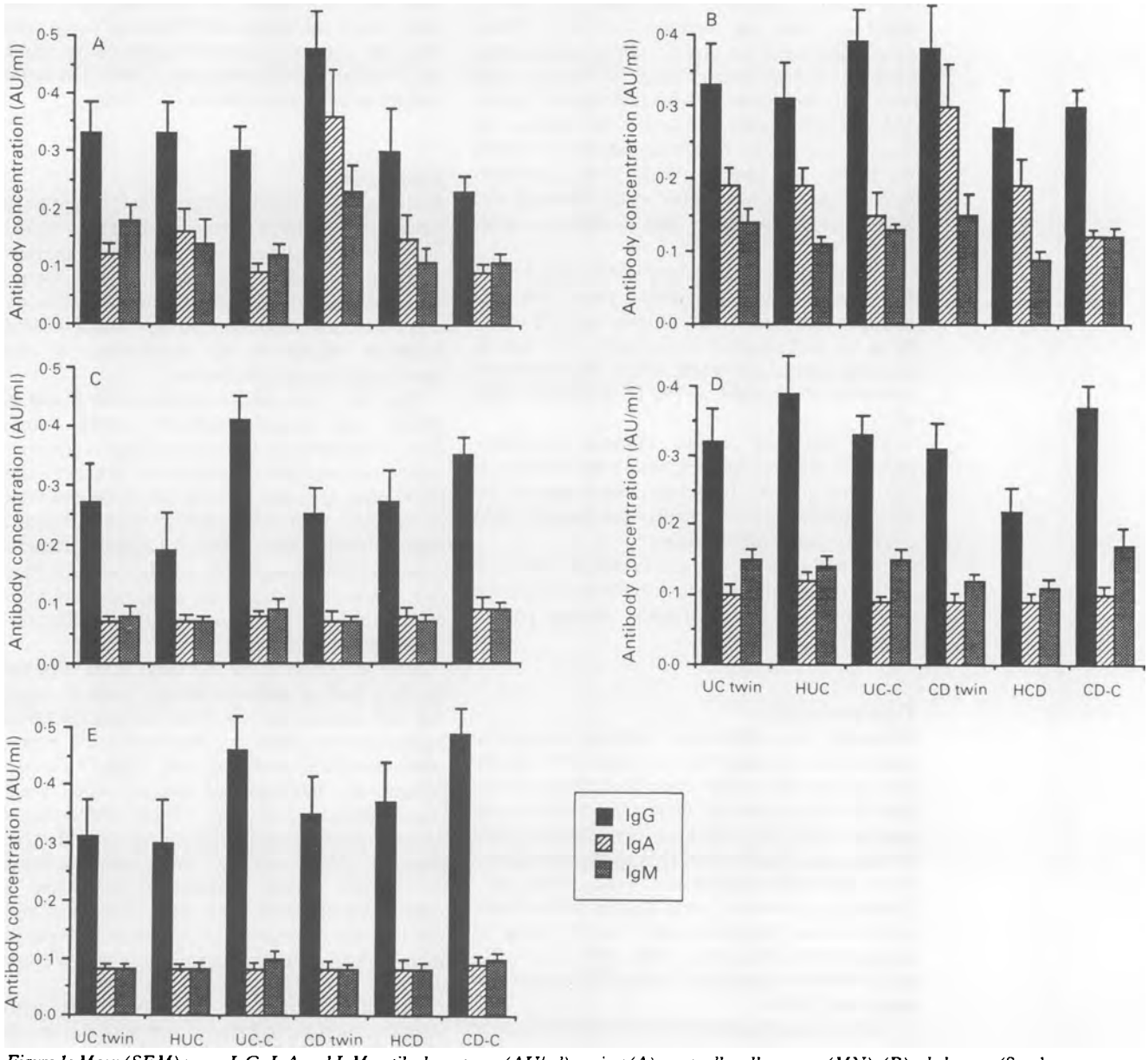


Figure 1: Mean (SEM) serum IgG, IgA and IgM antibody-response (AU/ml) against (A) yeast cell wall mannan (MN), (B) whole yeast (Saccharomyces cerevisiae) *preparation (YT), (C) betalactoglobulin (BLG), (D) gliadin (GL), and (E) ovalbumin (OA) in patients with ulcerative colitis (UC twin), their healthy monozygotic twins (HUC), matched controls (UC-C), and in patients with Crohn's disease (CD twin), their healthy monozygotic twins (HCD) and matched controls (CD-C).*

TABLE *Summary of statistical evaluations of serum levels in healthy twins, twins with inflammatory bowel disease and healthy control. Only statistically significant differences are depicted*

Antigen	Mannan			Yeast			Betalactoglobulin			Gliadin			Ovalbumin		
Antibody	IgG	IgA	IgM	IgG	IgA	IgM	IgG	IgA	IgM	IgG	IgA	IgM	IgG	IgA	IgM
Comparison 1. 2.															
CD HCD															
CD CD-C	>>>	>>>	>>		>>>				<<<			<			<
HCD CD-C		>			>				<<	<					<
UC HUC			>*			>*				<*					
UC UC-C		>									>>				<
HUC UC-C		>					<				>>>				<

CD: Twin with Crohn's disease, HCD: Healthy twin of patient with Crohn's disease, CD-C: Healthy control of Crohn's disease and HCD, UC: Twin with ulcerative colitis, HUC: Healthy twin of patient with ulcerative colitis, UC-C: Healthy control of UC and HUC.
>=values in column 1 larger than in col 2.
<=values in column 2 smaller than in column 1.
Significances: < and > ($p<0.05$), << and >> ($p<0.01$), <<< and >>> ($p<0.001$).
*Indicates that Wilcoxon's sign rank test was used instead of Students *t* test.

SPECIFIC

Crohn's disease

As seen in the Table, Crohn's disease patients had significantly higher levels of all immunoglobulin classes towards yeast cell wall mannan. The healthy twins had only higher IgA and the difference was not striking ($p<0.05$). When comparing Crohn's disease twins and healthy Crohn's disease twins using Wilcoxon's sign rank test, the diseased twins had slightly higher IgA and IgM against yeast cell wall mannan, but the difference was barely significant ($p=0.069$ for both). IgA against whole yeast (*Saccharomyces cerevisiae*) was higher in the diseased and healthy twins ($p<0.001$ and $p=0.011$, respectively).

The proportion of patients who had higher IgG, IgA, and IgM against yeast cell wall mannan compared with controls were 12 of 19, 18 of 19, and 14 of 19 respectively. The corresponding figures for whole yeast (*Saccharomyces cerevisiae*) were eight of 19, 15 of 19 and eight of 19.

The antibody levels towards betalactoglobulin, gliadin, and ovalbumin were similar in the three groups. In a few cases controls displayed higher antibody levels – for example, IgM to betalactoglobulin (Table).

The two patients on lactose reduced diet had similar in antibody response to betalactoglobulin compared with the Crohn's disease patient group.

Ulcerative colitis

Although the differences between ulcerative colitis twins, healthy twin of patient with ulcerative colitis and healthy control of twins concordant and discordant for ulcerative colitis were in general small, the following observations could be made on a group basis. IgM antibodies against yeast cell wall mannan and whole yeast (*Saccharomyces cerevisiae*) were higher in ulcerative colitis twins compared with healthy twin of patient with ulcerative colitis twins ($p=0.014$ and $p=0.026$ respectively) using Wilcoxon's sign rank test (Table).

Both diseased and healthy twins were found to have higher IgA response to yeast cell wall mannan compared with healthy control of twins concordant and discordant for ulcerative colitis ($p=0.046$ and $p=0.031$ respectively, Table).

The most striking finding was for IgA to gliadin, where both healthy twin of patient with ulcerative colitis and ulcerative colitis twins had higher levels than healthy control of twins concordant and discordant for ulcerative colitis ($p=0.001$ and $p=0.012$ respectively). The healthy twins had even higher IgG than ulcerative colitis twins when analysing with Wilcoxon's sign rank test. The antibody response to betalactoglobulin and ovalbumin is shown in the Table. The twins reacted similarly to controls.

Discussion

Earlier studies of food antigens in inflammatory bowel disease have shown conflicting results. The findings in the present study do not support an aetiologic role in inflammatory bowel disease of cow's milk proteins as reflected by antibody titres to betalactoglobulin. Neither could we find evidence supporting the importance of the potent egg allergen ovalbumin.

High IgA titres against gliadin were found in healthy and diseased ulcerative colitis twins. This could indicate a subclinical and/or genetically determined gluten intolerance. An increased prevalence of coeliac disease has been observed in patients with inflammatory bowel disease, especially ulcerative colitis.[14,15] Clinical experience does not strongly support such an aetiologic link, however, and so far no good epidemiological studies of gluten intolerance in inflammatory bowel disease exist.

More important is the fact that Crohn's disease patients had increased antibody titres to yeast cell wall mannan for all tested immunoglobulin classes and the difference was statistically strong when compared with controls. Crohn's disease patients also had high IgA levels to whole yeast (*Saccharomyces cerevisiae*). These observations are remarkable as almost all patients had inactive disease. Also healthy monozygotic twins of Crohn's disease diseased patients had a raised level of IgA to yeast cell wall mannan and whole yeast (*Saccharomyces cerevisiae*) compared with controls but the significance was weaker (Table).

This is in accordance with Main *et al*[12] who found an increase in IgG and IgA antibody levels to a crude yeast preparation in Crohn's disease patients compared with ulcerative colitis patients, and healthy controls, which was regarded as a reaction to *Saccharomyces*

cerevisiae. In a later study the same group[13] described a significant increase in IgG against 11 of 12 specified strains of *Saccharomyces cerevisiae*. A reaction to *Candida albicans* was not seen. We have used a similar method to prepare a crude yeast antigen mixture but also analysed the specific response to mannan which is a cell wall component in yeasts. In our study, all but two Crohn's disease patients had inactive disease, but they had increased antibody levels to yeast cell wall mannan and whole yeast (*Saccharomyces cerevisiae*). Nothing was mentioned about disease activity in the studies by Main *et al*[12] and McKenzie *et al*.[13] Notable in the present study was the increase in IgA antibodies to yeast cell wall mannan and whole yeast (*Saccharomyces cerevisiae*) also in healthy twins, which might indicate a genetic predisposition.

In the present study more than 240 statistical calculations were made, which must be considered when evaluating the results. To avoid differences by chance a significance level of at least $p<0{\cdot}01$ should be required. The response towards yeast cell wall mannan in Crohn's disease twins was very distinct and significant increased levels of all immunoglobulins tested were found.

Whether yeast mannan is an aetiological agent in Crohn's disease remains to be proved. Alternatively, yeast cell wall mannan may mimic a high mannose containing molecule, which is the offending compound and towards which the primary antibody response is directed. Taking this speculation even further, one might look for aberrant glycosylation patterns in Crohn's disease resulting in autoantibodies to high mannose containing glycoconjugates. Because the immune reaction is specific for yeast cell antigen(s) and apparently distinct for Crohn's disease, the primary disorder is probably not increased permeation of any dietary antigen. The permeability disorder seen in Crohn's disease[17–19] is more likely an effect of the inflammatory process.

We are grateful to Mr Bertil Larsson for skilled technical assistance. This study was supported by Örebro County Research Committee, AB Pharmacia (Uppsala, Sweden) and the Professor Nanna Svartz Foundation (awarded to EL) and by the Swedish Medical Research Council (Project, No 6251), the Swedish Society against Rheumatism and King Gustaf Vth 80-year Foundation (awarded to K-EM).

1 Andresen AFR. Gastrointestinal manifestations of food allergy. *Med J Rec* 1925; **122**: 271–5.
2 Taylor KB, Truelove SC. Circulating antibodies to milk proteins in ulcerative colitis. *BMJ* 1961; ii: 924–9.
3 Taylor KB, Truelove SC, Wright R. Serologic reactions to gluten and cow's milk proteins in gastrointestinal disease. *Gastroenterology* 1964; **46**: 99–108.
4 Sewell P, Cooke WT, Cox EV, Meynell MJ. Milk intolerance in gastrointestinal disorders. *Lancet* 1963; **ii**: 1132–5.
5 Dudek B, Spiro HM, Thayer WR. A study of ulcerative colitis and circulating antibodies to milk proteins. *Gastroenterology* 1965; **49**: 544–7.
6 Jewell DP, Truelove SC. Circulating antibodies to cow's milk proteins in ulcerative colitis. *Gut* 1972; **13**: 796–801.
7 Falchuk KR, Isselbacher KJ. Circulating antibodies to bovine albumin in ulcerative colitis and Crohn's disease. Characterization of antibody response. *Gastroenterology* 1976; **70**: 5–8.
8 Lerner A, Rossi TM, Park B, *et al*. Serum antibodies to cow's milk proteins in pediatric inflammatory bowel disease. *Acta Paediatr Scand* 1989; **78**: 384–9.
9 Paganelli R, Pallone F, Montano S, *et al*. Isotypic analysis of antibody response to food antigen in inflammatory bowel disease. *Int Archs Allergy Appl Immunol* 1985; **78**: 81–5.
10 Knoflach P, Park BH, Cunningham R, *et al*. Serum antibodies to cow's milk proteins in ulcerative colitis and Crohn's disease. *Gastroenterology* 1987; **92**: 479–85.
11 McCaffery TD, Kraft SC, Rothberg RM. The influence of different techniques in characterizing human antibodies to cow's milk proteins. *Clin Exp Immunol* 1972; **11**: 225–34.
12 Main J, McKenzie H, Yeaman GR, Kerr MA, Robson D, Pennington CR, *et al*. Antibody to *Saccharomyces cerevisiae* (baker's yeast) in Crohn's disease. *BMJ* 1988; **297**: 1105–6.
13 McKenzie H, Main J, Pennington CR, Parratt D. Antibody to selected strains of *Saccharomyces cerevisiae* (baker's and brewer's yeast) and *Candida albicans* in Crohn's disease. *Gut* 1990; **31**: 536–8.
14 Gillberg R, Dotevall G, Åhrén C. Chronic inflammatory bowel disease in patients with coeliac disease. *Scand J Gastroenterol* 1982; **17**: 491–6.
15 Kitis G, Holmes GKT, Cooper BT, Thompson H, Allan RN. Association of coeliac disease and inflammatory bowel disease. *Gut* 1980; **21**: 636–41.
16 Gray JG. Antibodies to cow's milk in ulcerative colitis. *BMJ* 1961; ii: 1265–6.
17 Olaison G, Leanderson P, Sjödahl R, Tagesson C. Intestinal permeability to polyethyleneglycol 600 in Crohn's disease. Peroperative determination in a defined segment of the small intestine. *Gut* 1988; **29**: 196–9.
18 Katz KD, Hollander D, Vadheim CM, *et al*. Intestinal permeability in patients with Crohn's disease and their healthy relatives. *Gastroenterology* 1989; **97**: 927–31.
19 Hollander D. Crohn's disease – a permeability disorder of tight junction? *Gut* 1988; **29**: 1621–4.
20 Stenhammar L, Brandt Å, Wågemark J. A family study of coeliac disease. *Acta Paediatr Scand* 1982; **71**: 625–8.
21 Kjellman N-IM, Björkstén B, Hattevik G, Fälth-Magnusson K. Natural history of food allergy. *Ann Allergy* 1988; **61**: 83–7.
22 Tysk C, Lindberg E, Järnerot G, Floderus-Myrhed B. Ulcerative colitis and Crohn's disease in an unselected population of monozygotic and dizygotic twins. A study of heritability and influence of smoking. *Gut* 1988; **29**: 990–6.
23 Cederlöf R, Friberg L, Jonsson E, Kaij L. Studies on similarity diagnosis in twins with the aid of mailed questionnaires. *Acta Genet* 1961; **11**: 338–62.

Adv Ther (2009) 26(8):795-804.
DOI 10.1007/s12325-009-0057-y

ORIGINAL RESEARCH

Immunogenic Yeast-Based Fermentation Product Reduces Allergic Rhinitis-Induced Nasal Congestion: a Randomized, Double-Blind, Placebo-Controlled Trial

Mark A. Moyad · Larry E. Robinson · Julie M. Kittelsrud · Stuart G. Reeves · Susan E. Weaver · Aireen I. Guzman · Mark E. Bubak

Received: June 17, 2009 / Published online: August 12, 2009 / Printed: September 10, 2009

ABSTRACT

Introduction: Allergic rhinitis (AR) impacts around 25% of the worldwide population. However, cost, safety, and a high dissatisfaction rate with numerous conventional medications continues to be an issue in the largest patient surveys, due primarily to a lack of efficacy on nasal congestion. Our previously published randomized trial demonstrated a significant reduction in cold and flu-like symptoms, and a secondary potential observation of a decrease in nasal congestion with an oral yeast-derived compound; therefore, the objective of this study was to test the effects of this same product on nasal congestion and other notable AR symptoms. ***Methods:*** A 12-week, randomized, double-blind, placebo-controlled clinical trial of 96 healthy subjects with a recent clinically documented history of seasonal allergies and AR was conducted. Participants received once-daily supplementation with 500 mg of a dried, modified *Saccharomyces cerevisiae* oral fermentation product (EpiCor®, Embria Health Sciences, Ankeny, Iowa, USA) or placebo during the 12-week period of the highest recorded concentrations of total pollen counts for this Midwest geographic area. Clinical outcome measurements included in-clinic examinations, validated questionnaire and standard diary, and serologic analysis at baseline, 6 and 12 weeks. ***Results:*** During the highest pollen count period (weeks 1-6), EpiCor significantly reduced the mean severity of specific AR symptoms, including a significant reduction in nasal congestion (P=0.04), rhinorrhea (P=0.005), and a nonsignificant reduction in ocular discharge symptoms.

Mark A. Moyad (✉)
Director of Preventive & Alternative Medicine, University of Michigan Medical Center, Department of Urology, Ann Arbor, Michigan 48109-0330, USA.
Email: mmmoyad@aol.com, moyad@umich.edu

Larry E. Robinson
Vice President, Scientific Affairs, Embria Health Sciences, Ankeny, Iowa, USA

Julie M. Kittelsrud · Susan E. Weaver
Nurse Practitioner, Avera Research Institute, Sioux Falls, South Dakota, USA

Stuart G. Reeves
Director of Research and Development, Embria Health Sciences, Ankeny, Iowa, USA

Aireen I. Guzman
Statistical Consultant, Avera Research Institute, Sioux Falls, South Dakota, USA

Mark E. Bubak
Assistant Professor, Allergy, Department of Internal Medicine, USD Sanford School of Medicine, Sioux Falls, South Dakota, USA

A significantly (*P*=0.04) reduced total number of days with nasal congestion (12.5 fewer days) favored EpiCor compared with placebo, as did the nasal congestion section of the quality of life questionnaire (*P*=0.04). Subjects receiving the intervention also experienced significantly (*P*=0.03) higher salivary IgA levels. Adverse events were similar to placebo. ***Conclusion:*** This yeast-derived product appeared to be safe and efficacious, and should receive more clinical research with and without standard medications to reduce the impact of seasonal allergies, especially AR-induced nasal congestion.

Keywords: allergic rhinitis; dietary supplement; EpiCor®; nasal congestion; *Saccharomyces cerevisiae*; seasonal allergy

INTRODUCTION

Allergic rhinitis (AR) is a common condition in the United States and throughout the world with reported prevalence rates of at least 10%-25%, and in some countries as high as 20%-50%.[1-4] AR is the sixth most common chronic health condition in the US, occurring in 10%-30% of adults and up to 40% of children.[5-6] It is the most prevalent chronic allergic disorder and is one of the ten most common medical conditions documented in the ambulatory care setting.[7] Costs from direct care and medications are a minimum of $3 billion annually in the US, with almost 80% spent on prescription medications.[8] Workplace productivity losses per employee with AR surpass those for workers with diabetes, migraine, respiratory infection and depression.[9] AR causes 3.8 million days lost annually from school and work in the US alone.[9]

Seasonal AR has a current estimated prevalence of 40% and perennial AR affects at least 10%-20% of the population, but both types of AR have increased over the past 40 years.[4,10-12] It is estimated that 40% of AR patients actually have both seasonal and perennial symptoms.[4] However, these numbers may represent a gross underestimation of the problem, because as many as one third of the individuals with either condition do not seek medical attention.

AR is notable for producing rhinorrhea; sneezing; pruritus of the nose, eyes, ears, and palate; and nasal congestion.[1] However, data from two of the largest patient surveys demonstrated that nasal congestion is usually the primary and dominating of all the symptoms, the one that is most concerning and bothersome, and it is the principal symptom that leads to medical attention and over-the-counter (OTC) or prescribed interventions.[13,14] For example, nasal congestion has been known to increase the risk of sleep disturbances, lower quality of life scores, increase absenteeism, and reduce productivity in the workplace and school.[15-17] Patient surveys also suggest that nasal congestion is not adequately controlled by currently available medications.[13,14]

A once-daily oral immunogenic fermentation product (EpiCor®, Embria Health Sciences, Ankeny, Iowa, USA) and dietary supplement partially derived from *Saccharomyces cerevisiae* (*S. cerevisiae*) has previously demonstrated the potential for adjuvant immune enhancement in a randomized, double-blind, and placebo-controlled trial of vaccinated subjects for influenza.[18] Significant reductions occurred in both the incidence and duration of cold and flu symptoms. One notable finding in this clinical trial was the significant reduction in nasal congestion with this intervention compared with placebo.[18] This observation, along with previous laboratory and clinical findings,[19] suggested the potential for this product to provide immune balance and activity against some of the common symptoms of AR, especially nasal congestion. Any

intervention that is safe, competitively priced, and potentially effective for nasal congestion specifically, and also for other AR issues, would be of interest because of the burden of this condition and the high rate of dissatisfaction with the current treatment options.[20]

MATERIALS AND METHODS

Population and Study Design

Inclusion criteria were as follows: generally healthy male and female subjects who were willing to sign informed consent and participate in all study activities; 18 years or older; self-report and also tested positive (ARUP Laboratories, Salt Lake City, Utah, USA) for grass allergy, which is indicative of seasonal allergies in the upper Midwest; experienced at least nasal symptoms and/or ocular symptoms on a seasonal basis; females who were not breastfeeding; and females who were of childbearing potential if they tested negative for pregnancy at the time of screening based on a serum test, intended not to become pregnant during the study, and agreed to utilize a reliable method of birth control. A past history of asthma was permitted and a total of 12 participants (seven on the intervention, and five on placebo) fit this profile.

Exclusion criteria were as follows: immune dysfunction and/or utilizing a prescribed immunosuppressive medication; uncontrolled asthma; nasal polyps; use of an intranasal steroid spray 1 month or less prior to randomization or during the study; HIV-positive; abnormal laboratory values; females who were pregnant, breastfeeding, or planning to become pregnant during the study; history of drug abuse; unable or unwilling to comply with the study protocol (ingesting study interventions, blood draws, completing diaries, and medical visits); current participation in another research study; comorbidity/concomitant disease; allergies to yeast or yeast-derived products; and chronic sinusitis and/or recent (within the last 6 weeks) episode of acute sinusitis.

A double-blind, placebo-controlled trial of 500 mg of EpiCor, a dietary supplement, was conducted to evaluate seasonal allergy symptoms in subjects 18 years or older. The placebo was of similar shape, size, consistency, and smell compared to the intervention. Each participant was asked to attend five clinical visits over a 12-week time period. Visit 1 included informed consent, standard serum analysis, and grass allergy screening by standard skin testing and/or serum analysis. At visit 2, all subjects that tested positive for grass allergies (n=96) that were asymptomatic and in good health were randomized to 500 mg once-daily EpiCor (n=48) or placebo (n=48). A licensed pharmacist, independent from the trial, utilized a random and blinded numerical and sequential distribution method, and assigned each participant to the intervention or placebo group. Medical history and examination was conducted along with standard blood analysis, saliva, inclusion and exclusion criteria, and the clinically validated Rhinoconjunctivitis Quality of Life Questionnaire (RQLQ),[21,22] along with a standardized symptom and adverse events diary[22] that was given to all participants to be completed daily. The RQLQ has seven domains: activities, sleep, non-nose/eye symptoms, practical problems, nasal symptoms, eye symptoms, and emotional dimension.[21,22] Each domain inquires about quality of life of the participant with a specific reference to the past week. The responses range from 0 to 6, with 0 indicating not troubled by the symptom and 6 as extremely troubled by the symptom. A lower overall score per symptom or domain is tantamount to a better quality of life.

The daily diary given to participants included the most common nose and eye

allergy symptoms. Subjects rated the presence or absence of their individual symptom on a daily basis, using a standard scale of 0-3, with 0 indicating the absence of the symptom and 3 indicating the most severe experience of this symptom.[22] The nasal symptoms were congestion, rhinorrhea, and sneezing. The eye symptoms included discharge, wateriness, and pruritus. Analysis of symptoms was done on two variables: the mean severity of the symptom and the mean total number of days the subject experienced the symptom (primary endpoints). Severity was defined as the average rating for the symptom only when the subject experienced it; therefore responses of 0 were excluded. Number of days with the symptom was defined as the total number of days the subject experienced the symptom. The days did not need to be continuous.

Visit 3 included collection of saliva samples, serum, quality of life, and review of adverse events and information from symptoms and adverse events diary. Visit 4 and 5 were similar to the third visit but also included nasal smear data collection. There was an approximate 6-week time period between visit 2 and 3, and 3-week time period between visit 3 and 4, and between visit 4 and 5 (12-week total interventional duration). Pollen counts (low, medium, and high) were based on the number of grains of pollen per cubic meter over a 24-hour period from diverse sources (trees, grasses, weeds, and mold) specific to this Midwest region of the country, and were monitored from Pollen.com, which provides daily monitoring of total pollen counts for every region of the US, utilizing comprehensive data from several hundred monitoring stations (http://www.pollen.com/allergy-weather-forecast.asp).

Participants were permitted to utilize OTC and prescription allergy antihistamine/decongestant medications, with the exception of steroids (exclusion criteria), on an "as needed" basis for allergy symptoms. Subjects were asked to record medications in the study diary. This was taken into consideration for statistical analysis. Allowed medications utilized during the study included the following: loratadine, fexofenadine, certirizine, montelukast, diphenhydramine, desloratadine, and sudafed.

Statistical Measurements

A power analysis was conducted based on data reported from a previous clinical trial with this intervention,[19] and for 85% statistical power at a significance level of 0.05 the required subjects would be approximately 40-50 per group, which set the optimal goal for recruitment. The analysis of symptoms was done in two parts: part 1 used the time factor as a grouping variable, and part 2 considered pollen counts. Comparison of the intervention and placebo groups was accomplished with the Mann-Whitney *U* test procedure at alpha equal to 0.05. All statistical analyses were done using version 9.1 of the SAS software. A *P* value of <0.05 was considered statistically significant.

RESULTS

Mean age of the EpiCor (intervention) group was 39 (SD±11.5) years with a maximum age of 62 and a minimum age of 18 years. Mean age of the placebo group was 38 (SD±12.5) years with a maximum age of 70 and a minimum age of 21 years. A total of 49% of the participants were female. Smoking status was as follows: 61% were nonsmokers in the EpiCor group and 56% in placebo. No statistical differences were noted for any of the baseline characteristics between the intervention and placebo groups. All of the subjects were Caucasian with the exception of one African-American in the placebo group.

Groups were divided into the three clinical visit periods after the intervention or placebo was utilized, and included weeks 1-6, weeks 7-9, and weeks 10-13. A significant difference occurred in pollen counts during these three different time intervals. A comparison of weeks 1-6 and weeks 7-9 resulted in a *P* value of 0.007, indicating a significantly higher pollen count on weeks 1-6 versus 7-9. Comparison of weeks 1-6 and weeks 10-13 also resulted in a highly significant ($P<0.0001$) difference in favor of a greater pollen count for weeks 1-6. Comparing weeks 7-9 and 10-13 demonstrated a significantly ($P=0.03$) higher pollen count during weeks 7-9. When comparing weeks 1-6 and 7-13 there was a significantly ($P=0.001$) higher pollen count for weeks 1-6 compared with any other time interval, whether or not that time interval was grouped (weeks 7-13) or separated in time (7-9 or 10-13).

During weeks 1-6 subjects given the intervention demonstrated significantly less mean severity of nasal congestion ($P=0.04$) and running nose ($P=0.005$) (see Tables 1 and 2). The median severity for nasal congestion was 1.14 with the intervention compared with 1.33 with placebo, and for rhinorrhea it was 1.24 versus 1.53. Total number of days with nasal congestion significantly ($P=0.04$) favored EpiCor with a median of 16.5 days of nasal congestion compared with 29 days with the placebo. Similar results and significance levels occurred regardless if median or

Table 1. The mean and median severity of symptom comparisons between the intervention (EpiCor) and placebo group over the period of highest pollen counts (weeks 1-6).

	Mean severity		Median severity		
Symptom	Intervention group (SD)	Placebo group (SD)	Intervention group (IQR)	Placebo group (IQR)	*P* value
Nasal congestion	1.29 (0.33)	1.43 (0.37)	1.14 (0.54)	1.33 (0.56)	0.040
Rhinorrhea	1.38 (0.39)	1.61 (0.41)	1.24 (0.56)	1.53 (0.54)	0.005
Sneezing	1.63 (0.44)	1.66 (0.42)	1.63 (0.78)	1.85 (0.74)	0.320
Ocular discharge	1.26 (0.32)	1.29 (0.40)	1.14 (0.38)	1.20 (0.47)	0.350
Ocular wateriness	1.31 (0.32)	1.37 (0.48)	1.19 (0.30)	1.18 (0.48)	0.440
Ocular pruritus	1.44 (0.51)	1.43 (0.36)	1.31 (0.67)	1.39 (0.48)	0.280

IQR=interquartile range; SD=standard deviation.

Table 2. The mean and median total number of days with the specific symptom comparisons between the intervention (EpiCor) and placebo group over the period of highest pollen counts (weeks 1-6).

	Mean total days with symptom		Median total days with symptom		
Symptom	Intervention group (SD)	Placebo group (SD)	Intervention group (IQR)	Placebo group (IQR)	*P* value
Nasal congestion	17.21 (14.11)	23.05 (15.65)	16.50 (26.00)	29.00 (31.00)	0.04
Rhinorrhea	21.86 (15.90)	22.74 (16.19)	20.50 (31.00)	29.00 (30.00)	0.50
Sneezing	22.50 (15.15)	21.90 (15.09)	25.00 (28.00)	25.00 (29.00)	0.37
Ocular discharge	7.45 (12.00)	10.69 (12.36)	2.00 (8.00)	7.00 (16.00)	0.06
Ocular wateriness	13.50 (12.93)	13.07 (13.85)	8.50 (22.00)	6.50 (22.00)	0.35
Ocular pruritus	13.26 (12.59)	15.05 (15.14)	10.50 (23.00)	10.50 (32.00)	0.42

IQR=interquartile range; SD=standard deviation.

mean days were compared between groups for weeks 1-6. Other measured symptom parameters (sneezing, ocular discharge, ocular wateriness, and pruritus) did not reach statistical significance. The additional time intervals (weeks 7-9 and 10-13) demonstrated no significance in symptom severity or total days with symptoms when the intervention was compared with placebo, with the exception of rhinorrhea that statistically (P=0.03) favored the intervention compared with placebo over the entire duration of the study period.

The RQLQ demonstrated that the nasal symptom domain, which includes stuffiness/blocked nose, runny nose, sneezing, and postnasal drip was significantly (P=0.04) less or in favor of EpiCor compared with placebo at visit 3. The EpiCor group also experienced significantly (P=0.04) less irritability at visit 4 compared with placebo. No other differences were noted with the RQLQ.

Rescue medication was utilized a median of 1 day with the intervention and 2 days with placebo for a P value of 0.04 in favor of EpiCor. Weeks 7-9 and 10-13 demonstrated no significant difference between the intervention and placebo.

The median IgE levels for the group given the intervention were nonsignificantly lower compared with placebo throughout the study, but did not reach statistical significance from visit 2 to 5. No statistical difference occurred for basophil or eosinophil concentration between groups. Salivary IgA levels were significantly (P=0.03) higher for EpiCor compared with placebo throughout the duration of the study.

Nasal smear data were collected at visit 4 and 5 only as an addendum to the ongoing protocol, and revealed a significantly (P=0.05 and P=0.03) lower number of lymphocytes for EpiCor (median = 18 and 16) compared with placebo (median = 60 and 57) at visit 4 and at visit 5 respectively, but no difference in monocytes. A marginally significant (P=0.056) reduction in eosinophils was observed in the EpiCor group compared with placebo, and a significantly (P=0.01) larger number of neutrophils occurred for the EpiCor group at visit 4 only. A nonsignificant improvement in quality of life scores occurred between visits 2 and 3 and between visits 4 and 5 in favor of EpiCor.

There were no significant differences between the intervention and placebo in terms of adverse events or drop-outs. A total of 10 subjects terminated prematurely including seven for personal reasons, two in the placebo due to side effects, and one participant became pregnant during the trial. A total of eight participants were lost to follow-up and had incomplete data. A total of 78 subjects completed the trial with equal numbers in the intervention and placebo group. Data analysis was completed on participants that had complete information during each time period.

DISCUSSION

Individuals with AR consistently refer to nasal congestion as the most concerning and bothersome symptom, and the one they would most like to prevent or treat due to the impact it has on overall quality of life and/or day-to-day activities.[13,14,23,24] Nasal congestion has a multifactorial etiology that includes inflammatory, neural, and vascular contributions.[25] These multiple pathways probably contribute to the complexity of trying to apply one appropriate individual treatment and may be responsible for the high dissatisfaction rate of the currently available treatments.[13,20] The prevalence and negative impact of AR and nasal congestion, and the limited therapeutic satisfaction of currently available treatments suggests that there is a strong need for novel options for this condition.

Complementary and alternative medicines are utilized in a large number of individuals with AR, but evidence-based recommendations do not exist for numerous reasons including the lack of safety data, and most clinical trials have not been rigorous enough to provide an endorsement of a specific intervention.[26-29] For example, past studies lack appropriate methodology including lack of randomization, not controlled, and not blinded, with no objective quantitative measurement. Also, numerous herbal remedies in this category lack quality control data. Regardless, there is a strong suggestion that a complementary or integrative therapy for AR that proved to have adequate impact through a well-designed clinical trial would ultimately be a welcome addition to conventional medicine. There are several different compounds that have received clinical results, but the strength of the clinical trial design has been questioned as well as the lack of impact on the most severe symptoms of AR, for example nasal congestion. EpiCor has currently completed four clinical trials that have all demonstrated positive immune modulating effects including cold and flu-like symptom reduction and now partial amelioration of some of the more problematic AR-induced manifestations such as nasal congestion.[18,19]

Nasal congestion is the predominant late-phase symptom of AR and results from the infiltration of inflammatory cells such as lymphocytes (T-cells) and eosinophils into tissue, and the subsequent prolonged release of mediators such as histamine, leukotrienes, and prostaglandins.[30] The finding of a consistent significant reduction in nasal congestion favoring EpiCor that translated clinically into approximately 12 fewer days of this symptom is notable and is on par with past studies of conventional prescription medication.[20] Nasal congestion was also analyzed as a separate clinical symptomatic entity in our clinical trial, which is a profound strength of the design. Numerous past studies are limited because this specific symptom has only been a part of a total nasal symptom score and not analyzed as a separate symptom, which questions the true clinical impact of these medications.

The strengths of our study include the randomized, double-blind, placebo-controlled design and the observation of enhanced nasal congestion resolution with this immunogenic fermentation product noted during the period of the highest recorded pollen counts, one of the primary endpoints, which provides the most impressive and consistent finding and suggests that the clinical impact was not due to chance. The quality of life correlations and improvement (less nasal congestion and irritability), and the significant increase in salivary IgA levels consistently in favor of EpiCor also strengthen the clinical observations. Furthermore, the finding of less severe rhinorrhea, and a significantly lower lymphocyte and nonsignificantly lower eosinophil nasal smear count in the EpiCor group, along with an increase in IL-10 from previous studies suggests that this intervention is potentially impacting the early and late-phase response observed with AR.[19] Higher endogenous IL-10 levels for example are partially responsible for resolving inflammation via inhibition of eosinophilia, suppression of nitric oxide production, and is a common mechanism of action whereby steroid therapy and allergen-specific immunotherapy may demonstrate their respective clinical efficacy.[31] Two other indirect observations of potential clinical significance also need mentioning. First a consistent reduction of several points in blood pressure and a reduction in CRP have been observed in past studies with this intervention compared with placebo,[18,19] which may also serve as markers of efficacy of this intervention and the other anti-inflammatory pathways that may be targeted.

Past studies of conventional medicine with allergies and asthma have suggested similar benefits in these two general health areas with effective medications.[32,33] However, the safety of this immunogenic fermentation product is consistently similar or less than placebo, which is notable when compared with common medications for AR.

Limitations of this clinical trial also deserve mentioning. EpiCor was most effective during the highest pollen count periods, but there was no greater perceived benefit compared to placebo during the time period of low pollen counts. IgE levels, although lower, were not significantly different, but in fairness more effective conventional medicines such as prescribed nasal steroids inhibit abnormal seasonal elevations in serum levels of circulating IgE antibodies,[34,35] which was similar to what was observed in our trial. However, unlike nasal steroids,[35] there was no clinically relevant reduction in overall ocular symptoms with the intervention utilized in our study. Eye symptoms, as with most clinical outcomes, favored EpiCor but did not reach significance. Quality of life scores, although improved overall compared with placebo, also did not reach statistical significance. Nasal smears should have been collected at baseline for complete comparative analysis, but a budgetary issue did not permit this ideal scenario. This clinical study also focused on treatment, thus further research in subjects with perennial AR might provide more insight into the preventive capacity of this intervention and should be the subject of further studies. Although, it is possible that many of the seasonal AR participants have perennial AR, and this also exemplifies the challenge in the design of these trials. It is difficult to capture clinical efficacy with AR subjects when predicted timing of pollen concentrations is also an inexact science.

Regardless, the strength of the study design, and the sum of the positive data suggests a true clinical impact in our opinion, especially in the area of nasal congestion, which is the most meaningful clinical endpoint in AR outcome studies. It is important to remember that first-line therapy for AR is based on a medication's ability to resolve nasal congestion, which is why prescribed intranasal corticosteroids fit in this category.[36] However, a multi-modality approach in our opinion would seem to have a higher probability of success in this category because of the complex nature of this condition and the unusually high rate of therapeutic dissatisfaction. For example, a second-generation antihistamine that has efficacy against pruritus in combination with this current intervention and its congestion-reducing properties, would be one of many potential interesting future clinical trials. The unique dual (allergy, and cold and flu-like symptoms) perennial clinical therapeutic efficacy demonstrated from this and past randomized clinical trials also needs to be further emphasized,[18,19] along with the safety profile, because it would certainly provide an argument that this specific intervention could set a novel research standard in the dietary supplement milieu.

CONCLUSION

A once-daily immunogenic fermentation yeast-derived product (EpiCor), significantly reduced nasal congestion by approximately 12 fewer days, and reduced other common symptoms in individuals with AR during the time of highest documented pollen count periods of the year. This dietary supplement should be given more clinical attention as a potential immune modulating intervention for susceptible individuals with and without currently available effective OTC and prescription medications.

ACKNOWLEDGMENTS

This research (abstract) was presented April 22, 2009 at the Experimental Biology Annual Meeting in New Orleans, Louisiana, USA. Embria Health Sciences owns EpiCor, and provided funding for this study and the page charges for publication. Larry Robinson, PhD, and Stuart Reeves, PhD, are employees, and Mark Moyad, MD, MPH, is a paid research consultant, of Embria Health Sciences. None of the other authors have any financial interest in Embria Health Sciences. Julie Kittelsrud, CNP, and Susan Weaver, CNP, are employees of the Avera Research Institute. Aireen Guzman is a paid statistical consultant, and Mark Bubak, MD, was a paid research consultant of the Avera Research Institute.

REFERENCES

1. van Cauwenberge P, Bachert C, Passalacqua G, et al. Consensus statement for the treatment of allergic rhinitis. European Academy of Allergology and Clinical Immunology. Allergy. 2000;55:116-134.

2. Bauchau V, Durham SR. Prevalence and rate of diagnosis of allergic rhinitis in Europe. Eur Respir J. 2004;24:758-764.

3. Skoner DP. Allergic rhinitis: definition, epidemiology, pathophysiology, detection and diagnosis. J Allergy Clin Immunol. 2001;108:2-8.

4. Bousquet J, Van Cauwenberge P, Khaltaev N; Aria Workshop Group: World Health Organization. Allergic rhinitis and its impact on asthma. J Allergy Clin Immunol. 2001;108(suppl. 5):S147-S334.

5. D'Alonzo GE Jr. Scope and impact of allergic rhinitis. J Am Osteopath Assoc. 2002;102(suppl. 2):S2-S6.

6. McMenamin P. Costs of hay fever in the United States in 1990. Ann Allergy. 1994;73:35-39.

7. Schappert SM, Burt CW. Ambulatory care visits to physician offices, hospital outpatient departments, and emergency departments: United States, 2001-02. Vital Health Stat. 2006;13:1-66.

8. Stempel DA, Thomas M. Treatment of allergic rhinitis: an evidence-based evaluation of nasal corticosteroids versus nonsedating antihistamines. Am J Man Care. 1998;4:89-96.

9. Lamb CE, Ratner PH, Johnson CE, et al. Economic impact of workplace productivity losses due to allergic rhinitis compared with select medical conditions in the United States from an employer perspective. Curr Med Res Opin. 2006;22:1203-1210.

10. Dykewicz MS, Fineman S. Executive summary of joint task force practice parameters on diagnosis and management of rhinitis. Ann Allergy Asthma Immunol. 1998;81:463-468.

11. Crystal-Peters J, Crown WH, Goetzel RZ, Schutt DC. The cost of productivity losses associated with allergic rhinitis. Am J Manage Care. 2000;6:373-378.

12. US Department of Health and Human Services web site. Agency for Healthcare Research and Quality. Management of Allergic and Nonallergic Rhinitis: Summary. Evidence Report/Technology Assessment 54. May 2002. Available at: http://www.ahrq.gov/clinic/epcsums/rhinsum.htm. Accessed July 2009.

13. Shedden A. Impact of nasal congestion on quality of life and work productivity in allergic rhinitis: findings from a large online survey. Treat Respir Med. 2005;4:439-446.

14. Allergies in America: a landmark survey of nasal allergy sufferers: executive summary. Florham Park, NJ: Altana Pharma US, Inc; 2006. Available at: http://www.myallergiesinamerica.com. Accessed June 1, 2009.

15. Santos CB, Pratt EL, Hanks C, et al. Allergic rhinitis and its effect on sleep, fatigue, and daytime somnolence. Ann Allergy Asthma Immunol. 2006;97:579-586.

16. Sundberg R, Toren K, Hoglund D, et al. Nasal symptoms are associated with school performance in adolescents. J Adolesc Health. 2007;40:581-583.

17. Nathan RA. The burden of allergic rhinitis. Allergy Asthma Proc. 2007;28:3-9.

18. Moyad MA, Robinson LE, Zawada ET Jr., et al. Effects of a modified yeast supplement on cold/flu symptoms. Urol Nurs. 2008;28:50-55.

19. Jensen GS, Patterson KM, Barnes J, et al. A double-blind placebo-controlled, randomized pilot study: consumption of a high metabolite immunogen from yeast culture has beneficial effects on erythrocyte health and mucosal immune protection in healthy subjects. Open Nutr J. 2008;2:68-75.

20. Nathan RA. The pathophysiology, clinical impact, and management of nasal congestion in allergic rhinitis. Clin Ther. 2008;30:573-586.

21. Juniper EF, Guyatt GH. Development and testing of a new measure of health status for clinical trials in rhinoconjunctivitis. Clin Exp Allergy. 1991;21:77-83.

22. Juniper EF, Stahl E, Doty RL, et al. Clinical outcomes and adverse effect monitoring in allergic rhinitis. J Allergy Clin Immunol. 2005;115:S390-S413.

23. Stewart MG. Identification and management of undiagnosed and undertreated allergic rhinitis in adults and children. Clin Exp Allergy. 2008; 38:751-760.

24. Stokes J, Fenstad E, Casale TB. Managing impairment in patients with allergic rhinitis. Allergy Asthma Proc. 2006;27:12-16.

25. Rosenwasser L. New insights into the pathophysiology of allergic rhinitis. Allergy Asthma Proc. 2007;28:10-15.

26. Passalacqua G, Bousquet PJ, Carlsen K-H, et al. ARIA update: I-Systematic review of complementary and alternative medicine for rhinitis and asthma. J Allergy Clin Immunol. 2006;117:1054-1062.

27. Man LX. Complementary and alternative medicine for allergic rhinitis. Curr Opin Otolaryngol Head Neck Surg. 2009;17:226-231.

28. Guo R, Pittler MH, Ernst E. Herbal medicines for the treatment of allergic rhinitis: a systematic review. Ann Allergy Asthma Immunol. 2007;99:483-495.

29. Mainardi T, Kapoor S, Bielory L. Complementary and alternative medicine: herbs, phytochemicals and vitamins and their immunologic effects. J Allergy Clin Immunol. 2009;123:283-294.

30. Storms WW. Pharmacologic approaches to daytime and nighttime symptoms of allergic rhinitis. J Allergy Clin Immunol. 2004;114:S146-S153.

31. Ogawa Y, Duru EA, Ameredes BT. Role of IL-10 in the resolution of airway inflammation. Curr Mol Med. 2008;8:437-445.

32. Magen E, Yosefy C, Viskoper RJ, Mishal J, Treatment of allergic rhinitis can improve blood pressure control. J Hum Hypertens. 2006;20:888-893.

33. Qian FH, Zhang Q, Zhou LF, et al. High-sensitivity C-reactive protein: a predictive marker in severe asthma. Respirology. 2008;13:664-669.

34. Naclerio RM, Adkinson NF, Creticos PS, et al. Intranasal steroids inhibit seasonal increases in ragweed-specific immunoglobulin E antibodies. J Allergy Clin Immunol. 1993;92:717-721.

35. Naclerio R. Intranasal corticosteroids reduce ocular symptoms associated with allergic rhinitis. Otolaryngol Head Neck Surg. 2008;138:129-139.

36. Plaut M, Valentine MD. Clinical practice. Allergic rhinitis. N Engl J Med. 2005;353:1934-1944.

THE JOURNAL OF ALTERNATIVE AND COMPLEMENTARY MEDICINE
Volume 16, Number 2, 2010, pp. 213–218

DOI: 10.1089/acm.2009.0310

Immunogenic Yeast-Based Fermentate for Cold/Flu-like Symptoms in Nonvaccinated Individuals

Mark A. Moyad, M.D., M.P.H.,[1] Larry E. Robinson, Ph.D.,[2] Edward T. Zawada, Jr., M.D.,[3] Julie Kittelsrud, R.N., M.S.N., C.N.P.,[3] Ding-Geng Chen, Ph.D.,[4] Stuart G. Reeves, Ph.D.,[2] and Susan Weaver, R.N., M.S.N., C.N.P.[3]

Abstract

Background: The common cold has a profound impact on employee attendance and productivity. Seasonal influenza is responsible for approximately 200,000 hospitalizations and 36,000 deaths per year in the United States alone. Over-the-counter medication efficacy has been questioned, and seasonal vaccination compliance issues abound. Our previously reported randomized trial of an oral fermentation product found an adjuvant benefit for vaccinated individuals in terms of a significantly reduced incidence and duration of cold and flu-like symptoms.

Methods: A concurrent 12-week, randomized, double-blind, placebo-controlled clinical trial of 116 subjects with no recent history of seasonal influenza vaccination was conducted. Participants received once-daily supplementation with 500 mg of a dried modified *Saccharomyces cerevisiae* oral fermentate (EpiCor) or placebo. Clinical outcome measurements included periodic interval-based in-clinic examinations and serologic analysis at baseline, 6 weeks, and 12 weeks. Participants utilized a standardized self-report symptom diary.

Results: Subjects receiving the intervention experienced a statistically significant reduction in the incidence ($p = 0.01$), a nonsignificant reduction in duration ($p = 0.10$), and no impact on the severity ($p = 0.90$) of colds or flu-like symptoms, but a more favorable safety profile compared with subjects receiving placebo.

Conclusions: This nutritional-based fermentate appeared to be safe and efficacious in a unique at-risk population and should receive more clinical research as a potential method to reduce the incidence of cold and flu-like symptoms, in individuals with and without a history of influenza vaccination.

Introduction

The common cold and its impact on work place absenteeism is well recognized.[1] It has become the third most common reason for physician office visits behind that of only hypertension and the recommended well-infant/child examinations.[2] Seasonal influenza's impact on morbidity and mortality rates are more concerning. In the United States alone, an estimated 200,000 hospitalizations and 36,000 deaths are attributed each year to this virus.[3,4] The Center for Disease Control (CDC) recently expanded the influenza immunization recommendations to include children beyond the age of 6 months and most adults.[3]

Compliance with vaccination recommendations has not mirrored the concerns of most established medical organizations, including the CDC.[3] A recent report on health care workers in the United States demonstrated that only 33% had received the influenza vaccination,[5] which generally reflects the rate of compliance reported by practitioners around the world.[6] This may be a primary reason the public has yet to embrace the importance of the vaccine. Other direct and indirect reasons for the low compliance rate may include (1) a recently released report that most strains in the current 2007–2008 vaccine were ineffective in preventing the majority of the flu cases;[3] (2) past perceptions that the vaccine was of minimal value;[3] (3) recent reports of viral resistance to prescription medication;[7,8] (4) ongoing evidence to suggest that many over-the-counter (OTC) preventive methods and medications to treat potential symptoms have no clinical value, or no exemplary clinical data, or safety issues;[9–11] and (5) concerns over a mercury preservative in the existing vaccine supply.[3] However, ample evidence exists to at least refute

[1]Department of Urology, University of Michigan Medical Center, Ann Arbor, MI.
[2]Embria Health Sciences, Ankeny, IA.
[3]Avera North Central Kidney Institute, Sioux Falls, SD.
[4]Department of Mathematics & Statistics, South Dakota State University, Brookings, SD.

most of these controversial issues surrounding the efficacy and safety of vaccination.[12,13]

Regardless, other safe and clinically tested methods that can be utilized to improve the immune status of the general public would still seem to be of interest, which would include those individuals who choose not to comply with vaccine recommendations; those who delay their own access or do not have immediate access to the vaccine; or individuals in the well-documented 2-week maximal antibody-generating waiting period postvaccination.[3]

Additionally, the spectrum of cold and flu symptoms overlap,[14,15] and an intervention that could impact the incidence of one or both of these conditions would again be another option in the ongoing search for effective OTC preventive items.

An oral immunogenic fermentation product (EpiCor®, Embria Health Sciences, Ankeny, IA) partially derived from *Saccharomyces cerevisiae* (*S. cerevisiae*) has already demonstrated the potential for adjuvant immune enhancement in a randomized, double-blind, placebo-controlled trial of vaccinated subjects for influenza.[16] Significant reductions occurred in both the incidence and duration of cold and flu symptoms. This trial addressed and answered one of two primary questions with this once-daily OTC supplement intervention: the potential capacity to safely enhance an already effective conventional medicine or at least add something novel during the most susceptible time of the year to cold and flu-like conditions. In this current clinical trial, we report the findings of the second concurrent trial and primary question that needed to be answered and construed: the ability to display some immunogenic potential when utilized as a sole agent in individuals who chose not to be vaccinated for seasonal influenza.

Materials and Methods

All methods listed in this section have been previously well described and were identical to our previous adjuvant trial,[16] with the exception of the nonvaccinated status requirement for this current study. The age range was 18–76 years, and subjects were living in a metropolitan area of the rural Midwest. Individuals had to be in good general health, as reflected by a Charlson comorbidity score of 0 or 1,[17] and via a standard basic history and physical examination by the clinical and research staff. Exclusion criteria are noted in Table 1.

This 12-week, randomized, double-blind, placebo-controlled trial was conducted during the acute period of the year for cold and flu seasonal symptoms (January through March). Healthy individuals without a recent history of vaccination for seasonal flu (influenza) giving informed consent ($n = 116$) were screened to determine baseline standardized laboratory values including complete blood count, complete metabolic profile, and other serologic parameters. Subjects were randomized to one of two groups: 500 mg of the daily, oral fermentate product (EpiCor, $n = 58$) or placebo ($n = 58$) for 12 consecutive weeks. The placebo capsule was of an identical appearance, odor, and weight compared to the active intervention. Participants were instructed to ingest medications with the first meal of the day.

Subjects attended the research institute clinic at weeks 0 (baseline), 6, and 12, and were required to record cold and flu-like symptoms at home in a modified standardized diary provided by the research center.[18] An overview of the diversity of the symptoms provided in this diary was provided in a previous publication.[16] Symptoms in the diary were rated from 0 (no symptoms) to 10 (most severe symptoms) and included the following: headache, general aches/pains, fatigue, weakness, nasal stuffiness, nasal drainage, sore throat, cough, hoarseness, chest discomfort, chills, fever, and miscellaneous or other, which had to be specified by the participant.

Each periodic clinical visit included: a standard history and physical examination, serologic sampling, vital signs (blood pressure, heart rate, temperature, and weight), on-site completed Short Form 36,[19,20] and reviewed and summarized diary information. The clinical study was approved by the Institutional Review Board for Avera Health (Sioux Falls, SD).

Common cold was clinically defined as an upper respiratory tract infection of viral etiology consisting of one or more

Table 1. Exclusion Criteria Utilized Before Randomization in the Cold and Flu Study of the Oral Fermentate-Based Product Compared to Placebo

History of an influenza vaccination in the past 12 months
Diagnosed, managed, or treated immune abnormality
Current use of any immunosuppressive prescription or over-the-counter medication such as azathioprine, cyclosporine, and steroids
Current use of any antiviral medication including amantadine, oseltamivir, rimantadine, and zanamivir
HIV positive
ALT, AST, BUN, and/or creatinine laboratory values greater than 2 times the upper limit of normal
Females who are pregnant, breastfeeding, or who are planning to become pregnant during the study period
History of substance abuse
Moderate to severe co-morbidity or concomitant disease or condition (Charlson score of 2 or greater)
Allergies to yeast or any yeast-derived products
Environmental allergies requiring medication or allergy-based injection therapy
Vitamin, mineral, or nutrient deficiency that requires supplementation
Herbal or supplemental preparation use in any form or formulation such as echinacea, vitamin C, or zinc
Unable or unwilling to comply with the study protocol, including ingesting the study supplement or placebo, regular blood sampling, and completing the study diary
Current participation in another clinical research investigation of any kind

HIV, human immunodeficiency virus; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen.

of the following symptoms: cough, generalized malaise, headache, hoarseness, low-grade fever, nasal drainage, nasal stuffiness, and sore throat.[3,14,15] Influenza-like symptoms were clinically defined as a respiratory tract infection of viral etiology and acute onset, more severe than the common cold, and consisting of one or more of the following symptoms: chest discomfort, fever of 102°F–105°F, myalgia, nonproductive cough, prominent headache, rhinitis, and sore throat.[3,14,15] Cold and flu-like symptoms could clinically overlap or occur simultaneously. The incidence of cold or flu-like symptoms was defined as the number of clinical occurrences reported during the entire 12-week study period. Duration of symptoms was defined as the number of consecutive illness days, and severity was also recorded and defined by a scale from 0 to 10 (least to most) as described by the self-report diary. The primary objective of the clinical trial was to determine whether a once-daily dose of the nutritional intervention would reduce the incidence, duration, and/or the severity of the common cold or influenza-like symptoms in healthy human subjects with no recent history of seasonal influenza vaccination. The statistical analysis software used by the statistician was the R program (2.9.0), which can be reviewed and obtained from www.r-project.org Final statistical analysis on the outcomes utilized two-way analysis of variance with EpiCor and placebo as treatment factor, and all symptoms as another factor. Analysis of covariance was also utilized when adjusting for covariates.

Results

Baseline characteristics for the EpiCor and placebo group are shown in Table 2.

No statistical significance between baseline characteristics of either group was identified.

The intervention significantly ($p = 0.01$) reduced the incidence of the common cold or flu-like symptoms compared to placebo. A mean of 1.32 (95% confidence interval [CI] 1.25–1.39) versus 1.51 (95% CI 1.37–1.65) clinical events occurred between the intervention and placebo groups, and this result remained significant regardless of the separate or combined baseline status parameters. The intervention had a greater impact on reducing the overall risk or incidence of 10 of the 11 specific symptoms compared to placebo, with the exception of weakness. Duration was nonsignificantly ($p = 0.10$) reduced from 4.25 (3.54–4.96) to 3.59 symptom days (95% CI 3.14–4.03) compared to placebo, and this result was again similar, regardless of baseline status. Duration symptoms were also reduced compared to placebo for 9 of the 11 parameters, with the exception of chills and chest discomfort. Severity score was not impacted by the intervention compared to the placebo ($p = 0.90$), which was 3.57 (95% CI 3.26–3.89) to 3.60 (95% CI 3.3–3.89). Fever was not impacted compared to placebo for incidence, duration, or severity, and the event rate was low overall (16 events compared to 14 events).

No abnormalities were found with any of the laboratory serologic parameters when comparing the intervention at baseline, to the intervention at 12 weeks, or when comparing intervention to placebo. The intervention significantly reduced systolic ($p = 0.04$) and diastolic ($p = 0.01$) blood pressure compared to placebo by 4 and 3 mm Hg, respectively.

The compliance rate (number of capsules consumed over the study period) in the intervention versus the placebo group was similar, with approximately 90% of capsules consumed. The rate of reporting any adverse event(s) was 29% for EpiCor and 48% for the placebo group, which is a significant difference ($p = 0.02$). There were a total of 3 dropouts during the trial, 1 in the placebo and 2 in the supplement group. Dropout was due to the lack of subject compliance with the protocol. None of the dropouts was for medication-related issues (intervention or placebo).

Discussion

Seemingly never-ending myriad untested OTC options for the prevention and relief of cold and flu-like symptoms exist,[10] and in the United States approximately $3 billion annually is spent on cold preparations alone.[21,22] Another estimated $1 billion goes toward filling unnecessary antibiotics prescriptions for these viral etiologies.

The ongoing concern by the U.S. Food and Drug Administration over nonefficacious or unsafe remedies should continue to result in the need to enforce more stringent research criteria for commercial availability and health claims.[9–11] This has been highlighted recently in studies that are challenging some long-standing and widely available untested and unproven OTCs available to consumers in the United States with simple and cost-effective home remedies.[23,24] These results should continue to generate thoughts about evidence-based medicine or simply the lack of evidence in some areas of the OTC market.

Additionally, despite nationally based educational efforts, a large segment of the population continues to forgo the seasonal influenza or other effective vaccinations.[6,12,25] Knowledge of this documented discrepancy, its consequences, an appreciation of the spectrum of flu-like signs and symptoms and a lack of OTC data were sufficient reasons for our research team to design and implement this unique trial.

Table 2. Baseline Characteristics of the Intervention and Placebo Group

Baseline characteristic	*Intervention* (n = *58*)	*Placebo* (n = *58*)
Age (mean ± SD)	37.1 (±13.5)	39.6 (±13.0)
Age range (years)	18–94	20–71
BMI (mean ± SD)	26.9 (±5.8)	27.0 (±4.2)
Gender (% female)	57%	60%
Race (% white)	97%	97%
Smoking status—never/past (%)	83%	85%
Smoking status—current(%)	17%	15%

BMI, body–mass index.

S. cerevisiae and/or products resulting from its fermentation with various substrates seem to be an appropriate choice for immune maintenance because of a long and notable clinical history of safety and clinical efficacy.[26,27] For example, one of the largest randomized trials of dietary selenium supplementation for cancer prevention demonstrated overall significant reductions in total cancer incidence, colorectal, prostate, and lung carcinoma. This trial utilized a modified 500 mg *S. cerevisiae* tablet that included 200 μg of selenium, as opposed to selenium by itself.[28] A more recent large-scale randomized trial of a combination low-dose nutritional supplement intervention included 100 μg of selenium that was also *S. cerevisiae*-derived, and researchers found a significant reduction in the risk of cancer in men, including prostate cancer.[29,30] No changes in hormone levels, prostate-specific antigen, or insulin-like growth factor 1 were noted despite these clinical benefits.[30] These past observations, along with the observations from our two trials, suggests that perhaps other mechanisms exist whereby risk reduction is achieved.

EpiCor was developed by Embria Health Sciences, LLC, of Ankeny, IA, and is classified as a dietary supplement.[31] It consists of *S cerevisiae*, grown under anaerobic and nutritional stress, in association with the nutrients and metabolites present in the fermentation broth, and in combination desiccated into a powdered form.[16] Over the last 60 years, a commercial feed additive product for farm animals only, based on this proprietary technology, has been utilized to enhance immune function and to prevent disease. The human-modified version of this product (EpiCor) has recently been subjected to multiple laboratory safety, stability, and efficacy investigations. It has demonstrated general and specific anti-inflammatory properties and potential immune support in humans with the stimulation of B-lymphocytes and natural killer cells, [32] and significantly increased salivary immunoglobulin A levels from a preliminary open label study of 22 adults before this trial was initiated (data on file). The same yeast species utilized in our trial may harbor a unique immune-modulating capacity because it is also utilized as the principal harvesting system for the current hepatitis B vaccine (HBV).[33] HBV is prepared via harvesting surface antigen of hepatitis B from cell cultures of recombinant strains of *S. cerevisiae*. Taken together, the objective and subjective data, and the direct and indirect evidence from *S. cerevisiae*-based technology were of an appropriate credibility to attempt some initial stage of immune therapy in a real-world setting.

EpiCor contains a series of macronutrients including fatty acids, such as oleic acid, which is found in olive oil, for example, and also a variety of soluble and insoluble dietary fibers. It contains almost the entire series of B-vitamins and minerals, but it is also unique in terms of its concentration of phytosterols and phenolic compounds such as resveratrol. Many of these individual compounds are at least the recipient of beneficial laboratory and clinical studies in medicine and immunology,[34–38] but in the oral fermentate they may synergistically garner an immune-modulating potential that may have some clinical application.

The *S. cerevisiae*-derived fermentate in this trial was not only safe but seems to provide some positive or no cardiovascular changes including blood pressure reductions. Similar to the ongoing paradigm with prescription preventive medicines, a dietary supplement, in our opinion, needs to have demonstrated some measure of safety, especially no cardiovascular issues, before being considered in this OTC category.

The results of this trial preliminarily espouse the previous observations and data on this modified *S. cerevisiae* fermentation product.[16] Incidence was moderately reduced between 10% and 20%; however, a total of 11 of the 12 symptoms decreased with this intervention. Duration of symptoms also decreased, which translated into an almost entire day of symptomatic reduction when cold and flu-like symptoms occurred. Thus, in total, the immune-protective properties seem consistent and noteworthy.

The overall strengths of this current study, especially for a dietary supplement, are also numerous and noteworthy. Based on strict and accepted methodological scoring systems utilized to analyze past clinical trials,[39] our trial fulfilled the majority of these criteria, which included (1) the large number of participants; (2) randomization of group assignment; (3) maintenance of the double-blind or treatment allocation concealment; (4) baseline similarities of the groups; (5) a withdrawal/dropout rate or narrow confidence intervals unlikely to cause bias; (6) blinding of the outcome assessors; (7) and a predefined primary outcome measurement and result completely reported. In our opinion, these are features, which are not commonly observed in OTC product studies. In addition, the strict exclusion and inclusion criteria, and the real-world setting of utilizing a product in a nonvaccinated milieu further establish the integrity of the observations. The financial cost to conduct such a clinically robust trial is a further testimony to the investigative team and the manufacturer of this product. Our research team also found that once-a-day dosing is a benefit in terms of simplicity and compliance issues.

Limitations of this clinical trial should also receive attention. More frequent clinical visits, albeit costly, would have allowed for closer follow-up and more precise serologic observations. The standardized diary is an imperfect system of measure, but was reviewed with each visit. Additional immunologic plasma, serum, urine, and imaging studies could have further enhanced the accuracy of the trial, including the duration and severity data. However, it should be reiterated that the monitoring of primarily symptoms in the case of colds and flu-like conditions remains the accepted standard for primary outcome measures utilized in conventional medical prescription drug and vaccine trials.[40] It would have also been advantageous to have information on workplace or household contacts who have been vaccinated that could potentially provide a herd immunity effect or a potential immunologic shield for a clinical trial participant. Randomization should have provided balance in terms of this concern, and it was reassuring to find no significant difference in baseline health characteristics among the intervention and placebo groups. Nevertheless, our recruitment methods failed to attract a diversity of participants in terms of minority group participation. This needs to be addressed and amended in future trials. Finally, an intent-to-treat design was not utilized, similar to the previous trial,[16] but only 3 participants dropped out of this study, and the overall methodology along with this compliance rate and consistency in the findings from the past and current trial should be sufficient, in our opinion, to ensure confidence in the results with this intervention compared to placebo.

Conclusions

In conclusion, this randomized trial demonstrated that a modified *S. cerevisiae*-based oral immunogenic fermentate taken once daily is safe and significantly reduced the incidence, and nonsignificantly reduced the duration of cold and flu-like symptoms. This is now the second randomized, double-blind, and placebo-controlled trial to date to demonstrate the potential for this product to improve clinical endpoints in an otherwise healthy population, regardless of vaccine history. These studies should potentially also serve as at least minimal criteria, in our opinion, for the type of research needed to establish credibility in the OTC market for cold and flu-like symptom prevention.

Acknowledgments

The authors want to thank all of the individual volunteers and the participants of this important and time-consuming clinical trial. This study was supported by Embria Health Sciences, Ankeny, IA.

Disclosure Statement

M. Moyad is a clinical research consultant and D.-G. Chen is a statistical consultant for Embria Health Sciences. L. Robinson and S. Reeves are employees of Embria Health Sciences. E. Zawada (principal investigator), J. Kittelsrud, and S. Weaver have no conflicts of interest.

References

1. National Institute of Allergy and Infectious Diseases. Common Cold. Online document at: www3.niaid.nih.Gov/healthscience/healthtopics/colds Accessed February 15, 2008.
2. Cherry DK, Woodwell DA, Rechtsteiner EA. National Ambulatory Medical Care Survey: 2005 Summary. Adv Data 2007;387:1–39.
3. Centers for Disease Control and Prevention. The 2007–2008 Flu Season. Online document at: www.cdc.gov/flu/about/qa/season.htm Accessed March 5, 2008.
4. Centers for Disease Control and Prevention. Prevention and control of influenza: Recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR 2006;55(RR10):1–42.
5. Fiore AE, Shay DK, Haber P, et al. Prevention and control of influenza: Recommendations of the Advisory Committee on Immunization Practices (ACIP), 2007. MMWR Recomm Rep 2007;56:1–54.
6. McLennan S, Gillett G, Celi LA. Healer, heal thyself: Health care workers and the influenza vaccination. Am J Infect Control 2008;36:1–4.
7. Hatakeyama S, Sugaya N, Ito M, et al. Emergence of influenza B viruses with reduced sensitivity to neuraminidase inhibitors. JAMA 2007;297:1435–1442.
8. Moscona A, McKimm-Breschkin J. News about influenza B drug resistance that cannot be ignored. JAMA 2007;297:1492–1493.
9. Aiello AE, Larson EL, Levy SB. Consumer antibacterial soaps: Effective or just risky? Clin Infect Dis 2007;45(suppl 2):S137–S147.
10. Guo R, Pittler MH, Ernst E. Complementary medicine for treating or preventing influenza or influenza-like illness. Am J Med 2007;120:923–929.
11. Kuehn BM. Citing serious risks, FDA recommends no cold and cough medicines for infants. JAMA 2008;299:887–888.
12. Andre FE, Booy R, Bock HL, et al. Vaccination greatly reduces disease, disability, death and inequity worldwide. Bull WHO 2008;86:140–146.
13. Nichol KL, Nordin J, Mullooly J, et al. Influenza vaccinations and reductions in hospitalizations for cardiac disease and stroke among the elderly. NEJM 2003;348:1322–1332.
14. Merck Manual of Medical Information. Second home edition. Beers MH, ed.-in-chief. New York: Pocket Books, 2004: 1154–1175.
15. Centers for Disease Control & Prevention. Questions & Answers: Cold Versus Flu. Online document at: www.cdc.gov/flu/about/qa/coldflu.htm Accessed March 1, 2008.
16. Moyad MA, Robinson LE, Zawada ET Jr, et al. Effects of a modified yeast supplement on cold/flu symptoms. Urol Nurs 2008;28:50–55.
17. Charlson M, Szatrowski TP, Peterson J, Gold J. Validation of a combined comorbidity index. J Clin Epidemiol 1994;47:1245–1251.
18. McDowell I. Measuring Health: A Guide to Rating Scales and Questionnaires. 3rd ed. New York: Oxford University Press, 2006:480–482.
19. Ware JE, Sherbourne CD. The MOS 36 item short-form health survey (SF-36). 1: Conceptual framework and item selection. Med Care 1992;30:473–480.
20. Patel AA, Donegan D, Albert T. The 36-item short form. J Am Acad Orthoped Surg 2007;15:126–134.
21. Fendrick AM, Monto AS, Nightengale B, Sarnes M. The economic burden of non-influenza related viral respiratory tract infection in the United States. Arch Intern Med 2003;163:487–494.
22. Roxas M, Jurenka J. Colds and influenza: A review of diagnosis and conventional, botanical, and nutritional considerations. Alt Med Rev 2007;12:25–48.
23. Paul IM, Beiler J, McMonagle A, et al. Effect of honey, dextromethorphan, and no treatment on nocturnal cough and sleep quality for coughing children and their parents. Arch Pediatr Adolesc Med 2007;161:1140–1146.
24. Warren MD, Pont SJ, Barkin SL, et al. The effect of honey on nocturnal cough and sleep quality for children and their parents. Arch Pediatr Adolesc Med 2007;161:1149–1153.
25. Hampton T. Research reveals low immunization rates and vaccination awareness among adults. JAMA 2008;299:1007.
26. Moyad MA. Brewer's/Baker's yeast (*Saccharomyces cerevisiae*) and preventive medicine: Part I. Urol Nurs 2007;27:560–561.
27. Moyad MA. Brewer's/Baker's Yeast (*Saccharomyces cerevisiae*) and preventive medicine: Part II. Urol Nurs 2008;28:73–74.
28. Clark LC, Combs GF Jr, Turnbull BW, et al. Effects of selenium supplementation for cancer prevention in patients with carcinoma of the skin: A randomized controlled trial. Nutritional Prevention of Cancer Study Group. JAMA 1996;276:1957–1963.
29. Hercberg S, Galan P, Preziosi P, et al. The SU.VI.MAX study: A randomized, placebo-controlled trial of the health effects of antioxidant vitamins and minerals. Arch Intern Med 2004;164:2335–2342.
30. Meyer F, Galan P, Douville P, et al. Antioxidant vitamin and mineral supplementation and prostate cancer prevention in the SU.VI.MAX trial. Int J Cancer 2005;116:182–186.
31. United States (U.S.) Food and Drug Administration Center for Food Safety and Applied Nutrition. Dietary

Supplements. Online document at: www.cfsan.fda.gov/~dms/ds-oview.html#what Accessed December 1, 2007.
32. Jensen GS, Hart AN, Schauss AG. An anti-inflammatory immunogen from yeast culture induces activation and alters chemokine receptor expression on human natural killer cells and B lymphocytes in vitro. Nutr Res 2007;27:327–335.
33. DiMiceli L, Pool V, Kelso JM, et al. and V.A.E.R.S. Team. Vaccination of yeast sensitive individuals: Review of safety data in the US vaccine adverse event reporting system (VAERS). Vaccine 2006;24:703–707.
34. Escrich E, Moral R, Grau L, et al. Molecular mechanism of the effects of olive oil and other dietary lipids on cancer. Mol Nutr Food Res 2007;51:1279–1292.
35. Vos A, M'Rabet L, Stahl B, et al. Immune modulatory effects and potential working mechanism of orally applied non-digestible carbohydrates. Crit Rev Immunol 2007;27:97–140.
36. Kirkland JB. Niacin and carcinogenesis. Nutr Cancer 2003; 46:110–118.
37. Bradford PG, Awad AB. Phytosterols as anticancer compounds. Mol Nutr Food Res 2007;51:161–170.
38. Orallo F. Comparative studies of the antioxidant effects of cis- and trans-resveratrol. Curr Med Chem 2006;13:87–98.
39. Jadad AR, Moore RA, Carroll D, et al. Assessing the quality of reports of randomized clinical trials: Is blinding necessary? Control Clin Trials 1996;17:1–12.
40. Eccles R. Understanding the symptoms of the common cold and influenza. Lancet Infect Dis 2005;5:718–725.

Address correspondence to:
Mark A. Moyad, M.D., M.P.H.
Department of Urology
University of Michigan Medical Center
Ann Arbor, MI 48109

E-mail: moyad@umich.edu

This article has been cited by:

1. Qiukui Hao, Zhenchan Lu, Bi Rong Dong, Chang Quan Huang, Taixiang Wu, Bi Rong DongProbiotics for preventing acute upper respiratory tract infections . [CrossRef]
2. Gitte S. Jensen , Kimberlee A. Redman , Kathleen F. Benson , Steve G. Carter , Marcie A. Mitzner , Stuart Reeves , Larry Robinson . 2011. Antioxidant Bioavailability and Rapid Immune-Modulating Effects After Consumption of a Single Acute Dose of a High-Metabolite Yeast Immunogen: Results of a Placebo-Controlled Double-Blinded Crossover Pilot Study. *Journal of Medicinal Food* **14**:9, 1002-1010. [Abstract] [Full Text] [PDF] [PDF Plus]

MAJOR ARTICLE

Saccharomyces cerevisiae Fungemia: An Emerging Infectious Disease

Patricia Muñoz,[1] Emilio Bouza,[1] Manuel Cuenca-Estrella,[3] Jose María Eiros,[1] Maria Jesús Pérez,[2] Mar Sánchez-Somolinos,[1] Cristina Rincón,[2] Javier Hortal,[2] and Teresa Peláez[1]

Departments of [1]Clinical Microbiology and Infectious Diseases and [2]Heart Surgery, Hospital General Universitario "Gregorio Marañón," Universidad Complutense, and [3]Centro Nacional de Microbiología, Instituto de Salud Carlos III, Madrid, Spain

(See the editorial commentary by Herbrecht and Nivoix on pages 1635–7)

Background. *Saccharomyces cerevisiae* is well known in the baking and brewing industry and is also used as a probiotic in humans. However, it is a very uncommon cause of infection in humans.

Methods. During the period of 15–30 April 2003, we found 3 patients with *S. cerevisiae* fungemia in an intensive care unit (ICU). An epidemiological study was performed, and the medical records for all patients who were in the unit during the second half of April were assessed.

Results. The only identified risk factor for *S. cerevisiae* infection was treatment with a probiotic containing *Saccharomyces boulardii* (Ultralevura; Bristol-Myers Squibb). This probiotic is used in Europe for the treatment and prevention of *Clostridium difficile*–associated diarrhea. The 3 patients received the product via nasograstric tube for a mean duration of 8.5 days before the culture result was positive, whereas only 2 of 41 control subjects had received it. Surveillance cultures for the control patients admitted at the same time did not reveal any carriers of the yeast. Strains from the probiotic capsules and the clinical isolates were identified as *S. cerevisiae*, with identical DNA fingerprinting. Discontinuation of use of the product in the unit stopped the outbreak of infection. A review of the literature identified another 57 cases of *S. cerevisiae* fungemia. Overall, 60% of these patients were in the ICU, and 71% were receiving enteral or parenteral nutrition. Use of probiotics was detected in 26 patients, and 17 patients died.

Conclusions. Use of *S. cerevisiae* probiotics should be carefully reassessed, particularly in immunosuppressed or critically ill patients.

Saccharomyces cerevisiae is a well-known yeast used in the food industry. It has now been demonstrated that this yeast can cause different forms of invasive infection [1–3], frequently after administration as a probiotic for the treatment of antibiotic-related diarrhea [4]. We report an outbreak of *S. cerevisiae* fungemia in an intensive care unit (ICU) that was traced, by means of molecular methods, to the use of probiotics, and we review all cases of *S. cerevisiae* fungemia that have been reported in the literature.

Received 9 November 2004; accepted 25 January 2005; electronically published 25 April 2005.

Reprints or correspondence: Dr. Patricia Muñoz, Hospital General Universitario "Gregorio Marañón," Doctor Esquerdo 46, 28007 Madrid, Spain (pmunoz@micro.hggm.es).

Clinical Infectious Diseases 2005; 40:1625–34

1058-4838/2005/4011-0008$15.00

PATIENTS, MATERIALS, AND METHODS

Setting. Our hospital is a 1750-bed, tertiary care, referral, general teaching institution. The heart surgery ICU of our hospital is a 14-bed postsurgery unit for all adult patients who have undergone a major cardiac surgical procedure.

Study of the outbreak of infection. During the period of 15–30 April 2003, we detected 3 patients with *S. cerevisiae* fungemia in our ICU. The medical records for 41 patients who had been in the unit during the second half of April 2003 were reviewed, in accordance with an established protocol, for epidemiological data, presence of *Clostridium difficile*–associated diarrhea, and use of *Saccharomyces boulardii* probiotic. Feces and pharynx surveillance cultures for the 14 patients in the ICU were also performed when the outbreak of infection was detected to test for *S. cerevisiae* carriage. Capsules of the probiotic were obtained for culture.

Table 1. Characteristics of 60 patients with fungemia caused by *Saccharomyces cerevisiae*.

Patient	Age in years, sex	Underlying condition or risk factor	Parenteral or enteral nutrition received	IV catheter	ICU stay	Sb probiotic use	Time to fungemia, days	Previous antimicrobial therapy	Other positive culture results	Disease	Therapy	Outcome	Reference(s)
1	54, F	Prosthetic valve endocarditis	NR	Yes	Yes	No	NA	Yes	Urine	Possible PVE	AmB	Survived	[10]
2	38, M	IDA, prosthetic tricuspid valve	NR	Yes	No	No	NA	Yes	No	Fungemia	AmB, surgery	Survived	[11, 12]
3	68, M	Ingestion of viable organism	No	No	No	No	NA	No	Urine, bone marrow	Disseminated	None	Survived	[13]
4	59, M	Burn	P	Yes	Yes	No	NA	Yes	Esophageal biopsy	Esophageal ulcer	AmB	Survived	[14]
5	61, M	RF, acute abdomen	NR	Yes	Yes	No	NA	Yes	CVC access site	Fungemia	Mico, 5FC	Died	[15]
6	37, F	HIV infection, RF, peritoneal dialysis	NR	Yes	NR	NR	NA	Yes	No	Fungemia	AmB	Survived	[18]
7	26, M	Nonneutropenic AML	NR	Yes	NR	NR	NR	No	No	Fungemia	NR	Survived	[17]
8	25, F	Multiple trauma	NR	Yes	Yes	No	NA	Yes	No	Fungemia	AmB	Survived	[18]
9	81, F	AML	NR	Yes	NR	NR	NR	NR	NR	Fungemia	AmB	Survived	[17]
10	62, F	Pancreatic cancer, hepatic abscesses	NR	Yes	NR	No	NA	Yes	Biliary fluid, liver abscess	Liver abscess	AmB	Died	[19]
11	65, F	Idiopathic pancytopenia, pneumonia, splenectomy, tuberculosis	NR	Yes	NR	No	NA	Yes	Lung biopsy, colon, heart, pericardium	Disseminated	None	Died	[19]
12	71, M	Aplastic anemia, neutropenia	NR	Yes	NR	No	NA	Yes	Throat	Fungemia	AmB, 5FC	Died (NRe)	[20]
13	0.1, NR	Abdominal surgery, respiratory failure	P	Yes	NR	No	NA	Yes	Urine	Fungemia	CVC removal	Survived	[20]
14	33, M	Ulcerative colitis, corticosteroid therapy, colectomy, RF	P/E	Yes	Yes	Yes	5	Yes	No	Fungemia	Flu, AmB	Survived	[21]
15	30, F	Allogeneic BMT	NR	Yes	NR	NR	NR	NR	No	Fungemia	NR	Cured	[22]
16	70, M	Myelodysplastic syndrome, tuberculosis	No	Yes	No	No	NA	Yes	No	Fungemia	None	Died	[23]
17	1, F	Bronchopneumonia	P	Yes	NR	Yes	13	Yes	No	Fungemia	Flu	Survived	[24]
18	14, M	Burn	E	Yes	Yes	Yes	102	Yes	No	Fungemia	5FC, AmB	Survived	[25]
19	NR	Prosthetic valve	NR	Yes	NR	NR	NA	Yes	NR	PVE	Surgery, AmB	Survived	[26]
20	48, F	BMT due to CML, GVHD	NR	Yes	No	No	NA	Yes	...	Fungemia	Flu	Survived	[27]
21	32, F	Self-inflicted fungemia; breast abscess	No	No	No	No	NA	No	No	Fungemia	...	Survived	[28]
22	16, M	Self-inflicted fungemia; convulsion	No	No	No	No	NA	No	No	Fungemia	...	Survived	[28]
23	34, M	Relapsed ALL	NR	Yes	No	No	NA	Yes: Flu	No	Fungemia	AmB	Died	[29]
24	49, M	Aspiration pneumonia	E	Yes	No	Yes	8	Yes	No	Fungemia	Flu	Survived	[30]
25	51, F	Polyarteritis nodosa (corticosteroids and cyclophosphamide therapy), CDAD	No	Yes	NR	Yes	18	Yes	No	Fungemia	AmB	Survived	[31]
26	10, F	Cystic fibrosis, bowel obstruction, biliar cirrhosis	P	Yes	No	Vicinity	NA	Yes	Postmortem lung, blood, mitral thrombus	Disseminated	AmB	Died	[32]
27	<1, M[a]	Congenital malformation	P	Yes	Yes	Vicinity	NA	Yes	No	Fungemia	AmB	Survived	[32]
28	7, M	Partial intestinal resection, parenteral nutrition	P	Yes		Vicinity	NA	Yes	No	Fungemia	AmB	Survived	[32]
29	85, M	Multiple trauma	NR	Yes	NR	NR	NA	Yes	No	Fungemia	AmB	Survived	[33]

30	78, M	COPD, diarrhea	E	Yes	Yes	Yes	21	Yes	No	Fungemia	Flu	Survived	[34]
31	<1, M[b]	Acute myeloid leukemia, neutropenia	No	Yes	No	Yes	NR	Yes: Flu	No	Fungemia	AmB	Survived	[35]
32	<1, M[c]	Congenital cardiopathy, diarrhea	P	Yes	Yes	Yes	10	Yes	No	Fungemia	L-AmB	Survived	[36]
33	<1, F[c]	Intestinal atresia	P	Yes	Yes	Vinity	NA	NR	No	Fungemia	AmB	Survived	[36]
34	74, M	Neurosurgery	E	No	Yes	Yes	NR	NR	No	Fungemia	Flu	Died	[37]
35	57, F	Prosthetic valve, endocarditis	No	No	No	No	NA	Yes	Aortic root abscess	PVE	Keto, partial surgical excision	Died	[38, 39]
36	2, M	Small bowel resection, cystic fibrosis	E	Yes	No	Yes	300	Yes	No	Fungemia	AmB	Survived	[40]
37	36, M	AIDS, lymphoma, MAID	P	Yes	No	Yes	7	Yes	No	Fungemia	Flu	Survived	[40]
38	47, M	Esophagectomy, pneumonia	P/E	Yes	No	Yes	10	Yes	Catheter	Fungemia	Flu	Survived	[40]
39	78, F	ARDS, peptic ulcer, RF	E	Yes	Yes	Yes	53	Yes	No	Fungemia	None	Survived	[40]
40	59, F	Cirrhosis, DM, peritonitis	P	Yes	Yes	No	NA	Yes	Vagina, RT, liver abscess	Disseminated	Flu, AmB	Died	[41]
41	<1, F[a]	Newborn	E	Yes	Yes	No	NA	Yes	No	Fungemia	AmB	Survived	[42]
42	<1, M[a]	Premature birth, ductus arteriosus, necrotizing enterocolitis	NR	Yes	Yes	No	NA	Yes	No	Endocarditis	AmB, Flu	Survived	[43]
43	56, M	Aortic-femoral graft, aortoenteric fistula	NR	Yes	No	No	NA	Yes	Periaortic fluid	Aortic graft infection	AmB	Died	[44]
44	50, M	Cardiac arrest	E	Yes	Yes	Yes	10	NR	...	Fungemia	None	Died (NRe)	[45]
45	51, F	Aortic surgery, cachexia	E	Yes	Yes	Yes	9	NR	...	Fungemia	Flu	Died (NRe)	[45]
46	50, M	ARDS, gastric ulcus	E	Yes	Yes	No	1	NR	Catheter	Fungemia	Flu	Survived	[45]
47	82, F	Acute respiratory failure	E	Yes	Yes	Yes	12	NR	No	Fungemia	None	Survived	[45]
48	75, M	Acute respiratory failure	E	Yes	Yes	Yes	14	NR	No	Fungemia	None	Survived	[45]
49	77, M	Peritonitis, duodenal ulcus	E	Yes	Yes	Yes	4	NR	No	Fungemia	AmB	Died (NRe)	[45]
50	71, F	Cerebrovascular stroke	E	Yes	Yes	Yes	9	NR	No	Fungemia	None	Survived	[45]
51	34, M	Head trauma	E	Yes	Yes	Vicinity	NA	Yes	No	Fungemia	Flu	Survived	[3]
52	48, M	Cerebral aneurism	E	Yes	Yes	Vicinity	NA	Yes	No	Fungemia	Flu	Survived	[3]
53	75, F	Myocardial infarction	E	Yes	Yes	Vicinity	NA	Yes	Catheter	Fungemia	Flu	Survived	[3]
54	35, F	Multiple trauma	E	NR	NR	NR	NA	NR	NR	Fungemia	NR	Survived	[3]
55	42, F	Kidney/pancreas transplant	No	Yes	No	Yes	7	Yes	No	Fungemia	Flu	Survived	[46]
56	41, M	DM, RF, HIV infection, tuberculosis, syphilis	No	Yes	No	Yes	15	Yes	No	Fungemia	AmB	Survived	[46]
57	<1, M[a]	Parenteral nutrition	P	Yes	Yes	Yes	4	No	No	Fungemia, methemoglobinemia	L-AmB	Survived	[47]
58	76, F	DM, heart surgery	P/E	Yes	Yes	Yes	9	Yes	Catheter	PVE	Flu	Died	PR
59	72, F	Heart surgery	P/E	Yes	Yes	Yes	7	Yes	Catheter hub	Fungemia	None	Died	PR
60	74, F	Rheumatoid arthritis, heart surgery	P/E	Yes	Yes	Yes	8	Yes	No	Fungemia	Flu	Died	PR

NOTE. ALL, acute lymphocytic leukemia; AmB, amphotericin B; AML, acute myelogenous leukemia; ARDS, acute respiratory distress syndrome; BMT, bone marrow transplantation; CDAD, *Clostridium difficile*-associated diarrhea; CML, chronic myelogenous leukemia; COPD, chronic obstructive pulmonary disease; CVC, central venous catheter; DM, diabetes mellitus; E, enteral; 5FC, 5-fluorocytosine; Flu, fluconazole; GVHD, graft-versus-host disease; IDA, injection drug abuser; Keto, keotconazole; L-AmB, liposomal amphotericin B; MAID, *Mycobacterium avium* intracellulare disease; Mico, miconazole; NA, not applicable; NR, not reported; NRe, nonrelated; P, parenteral; PR, present report; PVE, prosthetic valve endocarditis; RF, renal failure; Sb, *Saccharomyces boulardii*.

[a] Seven months old.

[b] Eight months old.

[c] One month old.

Identification of fungal isolates and susceptibility testing. One isolate from each patient with *S. cerevisiae* fungemia and the strain recovered from the probiotic capsules (the 4 study strains) were sent to the Mycology Reference Laboratory of the National Center for Microbiology of Spain (Madrid) for definitive identification and susceptibility testing. The isolates were identified by routine morphological and physiological tests [5, 6].

Susceptibility testing was conducted strictly on the basis of recommendations proposed by the Antifungal Susceptibility Testing Subcommittee of the European Committee on Antibiotic Susceptibility Testing for testing of fermentative yeasts (AFST-EUCAST discussion document 7.1) [7]. *Candida parapsilosis* ATCC22019 and *Candida krusei* ATCC6258 were used as quality-control strains.

The antifungal agents used in the study were amphotericin B, flucytosine, fluconazole, itraconazole, and voriconazole. These agents were dissolved in 100% dimethylsulfoxide (Sigma Aldrich Quimica). The MICs were determined at 24 and 48 h. MICs were obtained by measuring the absorbance at 530 nm with an MRXII reader (Dynatech; Cultek). For amphotericin B, the MIC end points were defined as the lowest drug concentration exhibiting reduction in growth of $\geq$90%, compared with that of the control growth. For flucytosine and azole drugs, the MIC end point was defined as the lowest drug concentration exhibiting a reduction in growth of 50%.

Molecular typing studies. Typing studies were performed on the basis of previously described procedures [8, 9]. The PCR fingerprinting procedure, which included 3 different primers, was used for molecular typing. The phage M13 core sequence (5′-GAGGGTGGCGGTTCT-3′) and primers that targeted microsatellite sequences $(GTG)_5$ and $(GACA)_4$ were used to amplify the DNA of the different strains. Amplification reactions were done in accordance with methods described in the literature [8, 9]. Amplifications were performed in a thermal cycler (GenAmp PCR System 2700; Applied Biosystems). Each amplification was repeated at least twice to verify the presence or absence of the scored bands. The amplified products were electrophoresed through 1.3% agarose gels (Pronadisa) and were stained with ethidium bromide (Sigma Aldrich Química). After intensive washing with distilled water, gels were photographed under UV light. PCR profiles were analyzed visually and indexed by letters or numbers, and even a single mismatch led to a different letter or number code.

Eight other clinical isolates of *S. cerevisiae* were used as control organisms in molecular typing studies (i.e., control strains). All strains were recovered over a 7-year period (1997–2003) from 8 different Spanish hospitals. Each clinical isolate represented a single isolate from a patient. These control isolates were not geographically or temporally related.

Statistical analysis. Relationships between variables were evaluated using the χ^2 statistic for categorical variables, Student's t test for normally distributed continuous variables, and the Mann-Whitney U test for nonparametric comparisons. All comparisons were considered to be statistically significant if the P value was $\leq$.05. Statistical analysis was performed using the SPSS software system (SPSS).

RESULTS

Case Reports

During the period of 15–30 April 2003, a total of 3 patients admitted to the major heart surgery ICU at our institution were found to have blood cultures positive for *S. cerevisiae.* These cases are described briefly below and in table 1.

Patient 1. Patient 1 was a 72-year-old woman who had undergone heart surgery on 14 March 2003. She had a complicated postoperative period, with multiple bacterial infections and *C. difficile*–associated diarrhea, for which she received Ultralevura (Bristol-Myers Squibb) starting on 8 April. On 15 April, she developed *S. cerevisiae* fungemia. Further culture results were negative, and no antifungal therapy was provided. The patient unexpectedly died on 29 April.

Patient 2. Patient 2 was a 74-year-old woman with rheumatoid arthritis who was receiving corticosteroid therapy. She required emergency replacement of her prosthetic mitral valve, and after her operation, she experienced different nosocomial infections, which required treatment with broad-spectrum antimicrobials, and *C. difficile*–associated diarrhea, for which Ultralevura was prescribed starting on 16 April.

On 23 April 2003, the patient developed sepsis and *S. cerevisiae* fungemia. The patient was treated with fluconazole (400 mg q.d.). The results of tip catheter and urine cultures were negative. She died on 5 May of catheter-related *Enterococcus faecium* bacteremia.

Patient 3. Patient 3 was a 76-year-old woman who required a mitral valve replacement and 4-vessel coronary artery bypass grafting on 4 April 2003. Her postoperative course was complicated by a perioperative myocardial infarction. She experienced diverse nosocomial infections and *C. difficile*–associated diarrhea, and she started receiving Ultralevura on 22 April. On 30 April, she developed sepsis and persistent *S. cerevisiae* fungemia. The probiotic treatment was stopped, and fluconazole (400 mg q.d.) was administered. A transesophageal echocardiogram revealed a vegetation on the prosthetic valve. The patient died on 20 June after a CNS stroke.

Colonization Surveillance

The results of feces and pharynx surveillance cultures for the 14 patients admitted to the unit when the outbreak of infection was recognized were negative for *S. cerevisiae.* No case of asymptomatic carriage was detected.

Role of Ultralevura Probiotic

The 3 case patients were treated with a probiotic preparation before the fungemia (Ultralevura), whereas only 2 of 41 control patients admitted to the ICU during April 2003 had received it. Capsules of the probiotic Ultralevura contain lyophilized *S. boulardii* and were opened and dissolved in the ICU before nasogastric administration. No further cases have been detected since the use of Ultralevura was discontinued in the unit.

The culture of the probiotic capsules showed heavy growth of a yeast (>1,000,000 cfu/mL) similar to that recovered from the 3 fungemic patients. All of the yeasts were identified as *S. cerevisiae.*

Results of Susceptibility and Molecular Typing Tests

The susceptibility results for the 4 *S. cerevisiae* study strains (1 from each patient and 1 from the probiotic batch) are shown in table 2. The molecular typing findings are shown in table 3. This table displays the genomic profile of the 4 study strains and of the other 8 *S. cerevisiae* isolates that acted as control organisms. Patterns obtained with primer M13 are shown in figure 1*A*, and profiles obtained with $(GACA)_4$ are shown in figure 1*B*. The most discriminative primer was the M13 sequence. This primer yielded 7 different patterns of bands for control organisms. Five patterns (A–E) were observed when $(GACA)_4$ was used, and 4 profiles *(a–d)* were observed with the $(GTG)_5$ sequence. When the results obtained with the 3 primers were combined, the 8 control isolates were differentiated, and each control strain was classified into a different genomic type. The profiles were reproducible between different DNA preparations from the same strain, as well as between runs when samples were run a second time.

With regard to the 4 study strains that were isolated from the probiotic and from the 3 temporally related patients, the genomic patterns were identical regardless of the typing technique utilized. This could indicate that the 4 isolates were epidemiologically related.

Review of the Literature about All Cases of S. cerevisiae Fungemia

We searched the MEDLINE database for articles in English, French, or Spanish that were published since 1966 using the medical subject headings "cerevisiae" "boulardii," and "fungemia." We searched reference lists to identify additional reports of *S. cerevisiae* fungemia. Cases with insufficient clinical information were excluded from this analysis [48–51]. Patients in whom the microorganism was not isolated from blood were also excluded [19, 52–56].

We were able to identify 60 cases of *S. cerevisiae* fungemia, including the 3 reported by our group in this article. The most important characteristics of these patients are presented in table 1.

The mean age ($\pm$SD) of the patients was 43 $\pm$ 26.8 years, and 7 patients were $\leq$1 year of age. Sex was reported for 57 patients, 31 (54.4%) of whom were male. Only 3 patients were healthy before the fungemia (2 patients with self-inflicted fungemia, who had infected themselves to escape from a prison camp, and 1 patient who ingested large quantities of brewer's yeast as a nutritional supplement). Overall, 28 (60%) of 47 patients whose data were reported were in the ICU when the fungemia was diagnosed, 35 (71%) of 49 were receiving enteral or parenteral nutrition, 55 (93%) of 59 had a central venous catheter in place, and 44 (88%) of 50 had received broad-spectrum antimicrobials. Results of catheter-tip cultures were positive for 6 patients (10%).

The use of probiotics was reported for 26 (45.6%) of 57 patients, and 5 other patients (9%) with fungemia were in the vicinity of patients receiving this therapy. The fungemia was detected a median ($\pm$SD) of 10 $\pm$ 62.3 days (range, 4–300 days) after the administration of the probiotic. Typing procedures were used in 39% of cases, and the capsules were cultured in 13%.

We compared patients who had and who had not received previous Ultralevura probiotic therapy. The only significant differences between the 2 groups were that patients who received probiotic therapy were more commonly in the ICU (70% vs 41%; $P = .05$), were more likely to have received parenteral or enteral nutrition (84% vs 50%; $P < .01$), and were less frequently treated with amphotericin B (32% vs 65%; $P = .01$).

The clinical presentation consisted of isolated fungemia (49 [82%] of 60 patients), endocarditis or periaortic abscess (5 [8.3%] of 60), disseminated disease (4 [6.7%] of 60), liver abscess (1 [1.7%] of 60), and esophageal ulcer (1 [1.7%] of

Table 2. Susceptibility test results for 4 epidemiologically related *Saccharomyces cerevisiae* strains isolated at a tertiary care hospital.

	MIC, μg/mL				
Strain ID	Amphotericin B	Flucytosine	Fluconazole	Itraconazole	Voriconazole
CNM-CL-5091	0.50	0.25	8.0	2.0	0.12
CNM-CL-5092	0.50	0.12	8.0	1.0	0.12
CNM-CL-5093	0.50	0.12	8.0	1.0	0.25
CNM-CL-5094	0.50	0.12	8.0	2.0	0.12

NOTE. CNM-CL, yeast collection of Spanish National Center for Microbiology.

Table 3. Molecular typing patterns for 4 epidemiologically related *Saccharomyces cerevisiae* strains isolated at a tertiary care hospital (study strains) and for 8 control strains.

Strain	Isolation site	Molecular typing pattern			Typing code	Genomic type
		M13	$(GACA)_4$	$(GTG)_5$		
Control strains						
CNM-CL-2780	Oropharyngeal exudate	1	A	*a*	1A*a*	I
CNM.CL-3831	Skin biopsy specimen	2	A	*b*	2A*b*	II
CNM-CL-4080	Bronchial secretion	3	B	*b*	3B*b*	III
CNM-CL-4238	Bronchial secretion	4	A	*b*	4A*b*	IV
CNM-CL-4256	Pleural fluid	1	B	*b*	1B*b*	V
CNM-CL-4456	Vaginal exudate	5	C	*a*	5C*a*	VI
CNM-CL-4542	Oropharyngeal exudate	6	D	*c*	6D*c*	VII
CNM-CL-4670	Blood	7	E	*d*	7E*e*	VIII
Study strains						
CNM-CL-5091	Ultralevura[a]	1	B	*b*	1B*b*	V
CNM-CL-5092	Blood	1	B	*b*	1B*b*	V
CNM-CL-5093	Blood	1	B	*b*	1B*b*	V
CNM-CL-5094	Blood	1	B	*b*	1B*b*	V

NOTE. Genomic profiles are indexed by letters or numbers.

[a] Manufactured by Bristol-Myers Squibb.

60). All but 12 patients received antifungal therapy (20%). The most common drugs received were fluconazole (16 patients) and amphotericin B (28 patients). The mortality rate was 28% (17 of 60 patients died). The only factor that increased the mortality rate was older age (mean age of patients who died and who survived, 60 years and 36 years, respectively; $P < .001$)

DISCUSSION

The extent of this review indicates that *S. cerevisiae* should be considered as a well-established cause of nosocomially acquired yeast infection, particularly in patients receiving prophylaxis or treatment with the probiotic Ultralevura *(S. boulardii),* which should be considered a risk factor for nosocomial bloodstream infection in patients with predisposing underlying conditions.

Saccharomyces is a ubiquitous ascomycetous yeast used by the food industry in the production of foodstuffs, wines, and beers. The identification of *S. cerevisiae* in the laboratory is not problematic and is usually based on the morphology of the yeast, its growth pattern, and biochemical studies (figure 1) [10].

The genus *Saccharomyces* includes several species, the most well-known being *S. cerevisiae.* Genotyping techniques, such as rDNA sequencing and random amplified polymorphic DNA analysis, have been used to identify isolates of *Saccharomyces* to the species level [36, 44, 48, 57]. After some discussion [58], *S. boulardii,* which is approved in many countries for the treatment or prevention of antibiotic-associated diarrhea, is now considered to be identical to a particular strain of *S. cerevisiae* [1, 46, 57]. This evidence is also supported by clinical studies such as ours, in which *S. cerevisiae* recovered from patients and *S. boulardii* strains isolated from probiotic preparations were proved to be genomically identical [3, 25, 30, 31, 36, 40, 45, 46].

The incidence of *S. cerevisiae* fungemia is unknown, although population-based studies suggest that it is responsible for 0.1%–3.6% of all episodes of fungemia [45, 48, 49]. The first case was reported in 1970 in a patient with a prosthetic mitral valve [10], and analysis of our review reveals an increase in the number of cases reported during the past decade (there were 4 cases reported during 1970–1980, 10 reported during 1981–1991, and 46 reported during 1992–2004).

S. cerevisiae is a common colonizer of mucosal surfaces and part of the normal flora of the gastrointestinal tract, the respiratory tract, and the vagina [59]. Its presence in normally sterile fluids has been classically described in patients with rupture of the local barriers or with very high fungal loads. Portals of entry include translocation of ingested microorganisms from the enteral or oral mucosa [24, 25, 37, 41, 51] and contamination of intravenous catheter insertion sites [40]. In our review, catheter tip culture results were positive in 6 cases (9.8%).

Hospital-acquired transmission has been demonstrated [3, 36, 45, 59], and both transmission from the environment and person-to-person transmission are possible [45, 52, 60]. *S. cerevisiae* fungemia may also be a self-inflicted disease [13, 28]. Finally, the difference in virulence between clinical and nonclinical strains may explain different degrees of invasiveness [1, 61, 62]

The most consistent risk factor for *S. cerevisiae* fungemia is the use of probiotics. Despite the fact that, in many cases, the

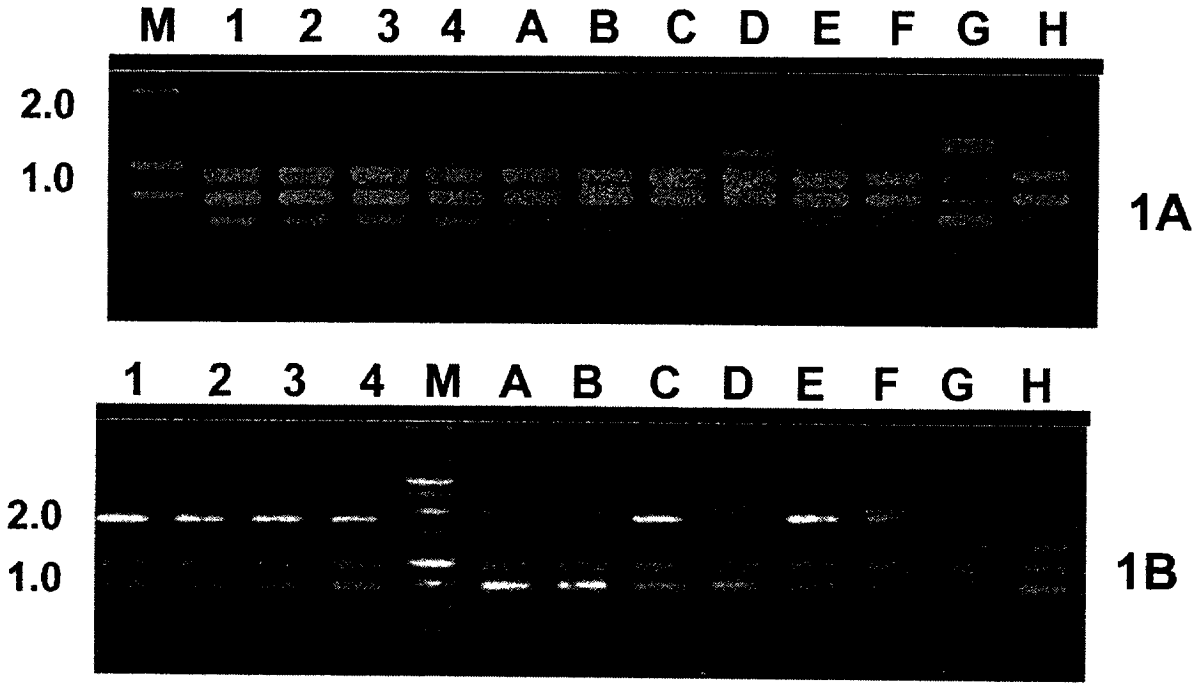


Figure. 1. PCR fingerprinting profiles obtained with primer M13 *(A)* and primer $(GACA)_4$ *(B). Lane M,* molecular marker (sizes in Kb), 1-kb ladder (Pharmacia; Madrid, Spain); *lanes A–H,* control strains (according to table 3); *lanes 1–4,* study isolates recovered from patients who were temporally related (according to table 3).

use of Ultralevura was not specified, 26 cases of *S. cerevisiae* fungemia—including ours—have been directly related to the oral administration of Ultralevura [15, 16, 18, 24, 25, 27, 30, 31, 34–37, 40, 45, 46]. In another 5 cases, the fungemic patients were reported to have been in the vicinity of probiotic-treated patients [3, 36, 45]. Fungemia was detected a median of 10 days after the administration of the probiotic (range, 4–300 days).

The use of probiotics may be especially dangerous in patients at high risk for infection. In fact, 15 of the 26 patients who developed fungemia after receipt of probiotic therapy were reported to be in the ICU, were receiving enteral feeding, had a central venous catheter in place, and were receiving antimicrobials. In these ICUs, at least 2 outbreaks (besides ours) have been described [3, 45]. It has been demonstrated that, when the probiotic capsules are opened for administration through the nasogastric tube, viable yeasts may be detected at a 1-m distance as a result of aerial transmission, and the yeasts persist on room surfaces after 2 h. They can be detected on the bare hands of health care workers even after vigorous hand washing [40]. In this setting, central venous catheters may be easily contaminated and become the portal of entry [40]. *S. cerevisiae* was recovered from the catheter hub of 1 of our patients, and catheter-related fungemia was further demonstrated by means of lysis centrifugation blood cultures.

As previously mentioned, classic severe immunosuppression is not a prerequisite for developing *S. cerevisiae* fungemia. In our review, 35 (71%) of the 49 patients with fungemia were receiving enteral or parenteral nutrition, 55 (93%) of 59 had a central venous catheter in place, and 44 (88%) of 50 had received broad-spectrum antimicrobials.

S. cerevisiae can cause a wide variety of clinical syndromes, such as pneumonia [19, 54], empyema [56], liver abscess [19], peritonitis [53, 63, 64], vaginitis [65–68], esophagitis [54, 69, 70], urinary tract infection [55, 71], cellulitis [72], unexplained fever, or septic shock [56]. *S. cerevisiae* has also been associated with Crohn disease, and the presence of antibodies against this microorganism is considered to be a sensitive (50%) and specific (90%) diagnostic test [73–76]. The microorganism has also been described in patients with asthma [77], ulcerative colitis, and diarrhea [78].

However, the most important clinical syndrome caused by *S. cerevisiae* is fungemia, because it is usually the most severe and well-proven clinical manifestation of the disease. *S. cerevisiae* fungemia has been described in immunosuppressed patients (19 patients [31%]) and critically ill patients (28 [46%]), but also in relatively healthy hosts. Underlying conditions include cancer [17, 19], HIV infection [16, 40, 46], use of corticosteroids [31, 78], neutropenia [19, 20, 29, 35], bone marrow transplantation [22, 27, 52], solid organ transplantation [46],

burns [14, 25], and heart surgery [10, 26, 35, 38, 43–45]. Critically ill neonates seem to be particularly predisposed to fungemia [20, 32, 33, 35, 36, 42, 43, 47].

Five patients with *S. cerevisiae* fungemia were found to have infectious endocarditis (including our case) [10, 26, 38, 39, 43]. Two of the 5 patients died. We have also identified a patient with infection of an aortic-bifemoral graft who developed aorto-enteric fistula and finally died of the infection [44].

Therapy for *S. cerevisiae* fungemia should rely on the withdrawal of the probiotic preparation, if it was being given, administration of an antifungal agent, and, as with other types of fungemia, withdrawal of central venous catheters [45, 48]. Persistent fungemia in patients with incomplete removal of prosthetic materials has been reported [32, 48].

The antifungal agent of choice has not been established. *S. cerevisiae* has been consistently susceptible to amphotericin B (MIC_{90}, 0.5–1 μg/mL) and fluorocytosine (MIC_{90}, 0.25 μg/mL), whereas different rates of resistance to fluconazole and itraconazole have been reported [41, 52, 79, 80]. The isolates exhibited in vitro resistance to itraconazole (MIC, 1–16 μg/mL), and the MIC_{90} to fluconazole was 8–128 μg/mL. We tested our 4 isolates and found that the most potent antifungal compounds in vitro were flucytosine and voriconazole, with MICs of ≤0.25 μg/mL. Besides, no therapeutic failure has been clearly attributable to resistance, even in strains with reduced susceptibility to fluconazole (MIC, 32 μg/mL) [40], although cases of fungemia occurred in patients receiving fluconazole or ketoconazole [29, 35].

Until more data are available, the antifungal agent of choice seems to be amphotericin B. However, this does not imply, in our opinion, the impossibility of treating patients with fluconazole, because most MICs obtained for this drug are within the susceptibility range, and many successful results obtained with fluconazole have been reported in the literature (table 1). Despite the fact that data are scarce, both posaconazole and voriconazole have been reported to have good in vitro activity against this fungus [81–85].

The mortality rate for patients with *S. cerevisiae* fungemia was 29.5% (18 patients), although, in most cases, mortality could not be only attributed to fungemia. However, septic shock has been reported in patients with *S. cerevisiae* fungemia as a single isolate [36, 40], and Piarroux et al. [48] described a patient who died of sepsis even though *Saccharomyces* was the only microorganism isolated from blood cultures.

S. cerevisiae should not be dismissed as a nonpathogenic microorganism when recovered from a clinical source. A history of intake of health food supplements or probiotics should be investigated.

Acknowledgments

Financial support. Supported in part by Red Española de Investigación en Patología Infecciosa (REIPI-C03/14).

Potential conflicts of interest. All authors: no conflicts.

References

1. McCullough MJ, Clemons KV, McCusker JH, Stevens DA. Species identification and virulence attributes of *Saccharomyces boulardii* (nom. inval.). J Clin Microbiol **1998**; 36:2613–7.
2. Eddy JT, Stamatakis MK, Makela EH. *Saccharomyces boulardii* for the treatment of *Clostridium difficile*–associated colitis. Ann Pharmacother **1997**; 31:919–21.
3. Cassone M, Serra P, Mondello F, et al. Outbreak of *Saccharomyces cerevisiae* subtype *boulardii* fungemia in patients neighboring those treated with a probiotic preparation of the organism. J Clin Microbiol **2003**; 41:5340–3.
4. Bleichner G, Blèhaut H, Mente H, Moyse D. *Saccharomyces boulardii* prevents diarrhea in critically ill tube-fed patients. Intensive Care Med **1997**; 23:517–23.
5. de Hoog GS, Guarro J, Gene J, Figueres MJ. Atlas of clinical fungi. Utrecht, The Netherlands and Reus, Spain: Centraalbureau voor Schimmelcultures/Universitat Rovira i Virgili, **2000**.
6. Kurtzman CP, Fell JW. The yeasts: a taxonomic study. Amsterdam: Elsevier, **1998**.
7. Rodriguez-Tudela JL, Barchiesi F, Bille J, et al. Method for the determination of minimum inhibitory concentration (MIC) by broth dilution of fermentative yeasts. Clin Microbiol Infect **2003**; 9:1–8.
8. Diaz-Guerra TM, Martinez-Suarez JV, Laguna F, Valencia E, Rodriguez-Tudela JL. Change in fluconazole susceptibility patterns and genetic relationship among oral *Candida albicans* isolates. AIDS **1998**; 12: 1601–10.
9. Gadea I, Cuenca-Estrella M, Prieto E, et al. Genotyping and antifungal susceptibility profile of *Dipodascus capitatus* isolates causing disseminated infection in seven hematological patients of a tertiary hospital. J Clin Microbiol **2004**; 42:1832–6.
10. Stein PD, Folkens AT, Hruska KA. *Saccharomyces* fungemia. Chest **1970**; 58:173–5.
11. Rubinstein E, Noriega ER, Simberkoff MS, Holzman R, Rahal JJ. Fungal endocarditis: analysis of 24 cases and review of the literature. Medicine (Baltimore) **1975**; 54:331–45.
12. Noriega ER, Rubinstein E, Simberkoff MS, Rahal JJ. Subacute and acute endocarditis due to *Pseudomonas cepacia* in heroin addicts. Am J Med **1975**; 59:29–36.
13. Jensen DP, Smith DL. Fever of unknown origin secondary to Brewer's yeast ingestion. Arch Intern Med **1976**; 136:332–3.
14. Eschete ML, West BC. *Saccharomyces cerevisiae* septicemia. Arch Intern Med **1980**; 140:1539.
15. Cimolai N, Gill MJ, Church D. *Saccharomyces cerevisiae* fungemia: case report and review of the literature. Diagn Microbiol Infect Dis **1987**; 8:113–7.
16. Sethi N, Mandell W. Saccharomyces fungemia in a patient with AIDS. N Y State J Med **1988**; 88:278–9.
17. Anaissie E, Bodey GP, Kantarjian H, et al. New spectrum of fungal infections in patients with cancer. Rev Infect Dis **1989**; 11:369–78.
18. Manzella JP, Shaffer S, Agarwal N, Kellogg JA. *Saccharomyces cerevisiae* fungemia in a multiply traumatized patient. J Trauma **1989**; 29:129–30.
19. Aucott JN, Fayen J, Grossnicklas H, Morrissey A, Lederman MM, Salata RA. Invasive infection with *Saccharomyces cerevisiae*: report of three cases and review. Rev Infect Dis **1990**; 12:406–11.
20. Nielsen H, Stenderup J, Bruun B. Fungemia with Saccharomycetaceae: report of four cases and review of the literature. Scand J Infect Dis **1990**; 22:581–4.

21. Zunic P, Lacotte J, Pegoix M, et al. *Saccharomyces boulardii* fungemia: apropos of a case [in French]. Therapie **1991**; 46:498–9.
22. Morrison VA, Haake RJ, Weisdorf DJ. The spectrum of non-*Candida* fungal infections following bone marrow transplantation. Medicine (Baltimore) **1993**; 72:78–89.
23. Oriol A, Ribera JM, Arnal J, Milla F, Batlle M, Feliu E. *Saccharomyces cerevisiae* septicemia in a patient with myelodysplastic syndrome. Am J Hematol **1993**; 43:325–6.
24. Pletincx M, Legein J, Vandenplas Y. Fungemia with *Saccharomyces boulardii* in a 1-year-old girl with protracted diarrhea. J Pediatr Gastroenterol Nutr **1995**; 21:113–5.
25. Viggiano M, Badetti C, Bernini V, Garabedian M, Manelli JC. *Saccharomyces boulardii* fungemia in a patient with severe burns [in French]. Ann Fr Anesth Reanim **1995**; 14:356–8.
26. Muehrcke DD, Lytle BW, Cosgrove DM 3rd. Surgical and long-term antifungal therapy for fungal prosthetic valve endocarditis. Ann Thorac Surg **1995**; 60:538–43.
27. Cairoli R, Marenco P, Perego R, de Cataldo F. *Saccharomyces cerevisiae* fungemia with granulomas in the bone marrow in a patient undergoing BMT. Bone Marrow Transplant **1995**; 15:785–6.
28. Fung KS, Scheel O, Lyon DJ, Cheng AF, Bendeck J. Self-inflicted bacteraemia and fungaemia in Vietnamese migrants. Scand J Infect Dis **1996**; 28:83–5.
29. Yoshida M, Obayashi T, Iwama A, et al. Detection of plasma (1→3)-β-D-glucan in patients with *Fusarium, Trichosporon, Saccharomyces* and *Acremonium* fungaemias. J Med Vet Mycol **1997**; 35:371–4.
30. Fredenucci I, Chomarat M, Boucaud C, Flandrois JP. *Saccharomyces boulardii* fungemia in a patient receiving Ultra-levure therapy. Clin Infect Dis **1998**; 27:222–3.
31. Bassetti S, Frei R, Zimmerli W. Fungemia with *Saccharomyces cerevisiae* after treatment with *Saccharomyces boulardii*. Am J Med **1998**; 105: 71–2.
32. Fiore NF, Conway JH, West KW, Kleiman MB. *Saccharomyces cerevisiae* infections in children. Pediatr Infect Dis J **1998**; 17:1177–9.
33. Pavese P, Brion JP, Lebeau B, Grillot R, Ambroise-Thomas P. Epidemiology of fungemia in a university hospital; therapeutic incidence [in French]. Pathol Biol (Paris) **1999**; 47:579–83.
34. Niault M, Thomas F, Prost J, Ansari FH, Kalfon P. Fungemia due to *Saccharomyces* species in a patient treated with enteral *Saccharomyces boulardii*. Clin Infect Dis **1999**; 28:930.
35. Cesaro S, Chinello P, Rossi L, Zanesco L. *Saccharomyces cerevisiae* fungemia in a neutropenic patient treated with *Saccharomyces boulardii*. Support Care Cancer **2000**; 8:504–5.
36. Perapoch J, Planes AM, Querol A, et al. Fungemia with *Saccharomyces cerevisiae* in two newborns, only one of whom had been treated with ultra-levura. Eur J Clin Microbiol Infect Dis **2000**; 19:468–70.
37. Rijnders BJ, Van Wijngaerden E, Verwaest C, Peetermans WE. *Saccharomyces* fungemia complicating *Saccharomyces boulardii* treatment in a non-immunocompromised host. Intensive Care Med **2000**; 26:825.
38. Ubeda P, Viudes A, Perez Belles C, Marques JL, Peman J, Gobernado M. Endocarditis por *Saccharomyces cerevisiae* sobre válvula protesica. Enferm Infecc Microbiol Clin **2000**; 18:142.
39. Ubeda P, Perez C, Blanes M, Viudes A, Peman J, Gobernado M. Endocarditis infecciosa por levaduras. Enferm Infecc Microbiol Clin **2001**; 19:500–2.
40. Hennequin C, Kauffmann-Lacroix C, Jobert A, et al. Possible role of catheters in *Saccharomyces boulardii* fungemia. Eur J Clin Microbiol Infect Dis **2000**; 19:16–20.
41. Heath CH, Jaksic A, McKerracher D, Clarke GM. Disseminated *Saccharomyces cerevisiae* infection following polymicrobial hepatobiliary sepsis. Aust N Z J Med **2000**; 30:521–2.
42. Ipson MA, Blanco CL. *Saccharomyces cerevisiae* sepsis in a 35-week-old premature infant: a case report. J Perinatol **2001**; 21:459–60.
43. Ruiz-Esquide F, Diaz MC, Wu E, Silva V. Verrucous endocarditis secondary to *Saccharomyces cerevisiae:* a case report [in Spanish]. Rev Med Chil **2002**; 130:1165–9.
44. Smith D, Metzgar D, Wills C, Fierer J. Fatal *Saccharomyces cerevisiae* aortic graft infection. J Clin Microbiol **2002**; 40:2691–2.
45. Lherm T, Monet C, Nougiere B, et al. Seven cases of fungemia with *Saccharomyces boulardii* in critically ill patients. Intensive Care Med **2002**; 28:797–801.
46. Riquelme AJ, Calvo MA, Guzman AM, et al. *Saccharomyces cerevisiae* fungemia after *Saccharomyces boulardii* treatment in immunocompromised patients. J Clin Gastroenterol **2003**; 36:41–3.
47. Lungarotti MS, Mezzetti D, Radicioni M. Methaemoglobinaemia with concurrent blood isolation of *Saccharomyces* and *Candida*. Arch Dis Child Fetal Neonatal Ed **2003**; 88:F446.
48. Piarroux R, Millon L, Bardonnet K, Vagner O, Koenig H. Are live saccharomyces yeasts harmful to patients? Lancet **1999**; 353:1851–2.
49. Rees JR, Pinner RW, Hajjeh RA, Brandt ME, Reingold AL. The epidemiological features of invasive mycotic infections in the San Francisco Bay Area, 1992–1993: results of population-based laboratory active surveillance. Clin Infect Dis **1998**; 27:1138–47.
50. Taylor GD, Buchanan-Chell M, Kirkland T, McKenzie M, Wiens R. Trends and sources of nosocomial fungaemia. Mycoses **1994**; 37: 187–90.
51. Debelian GJ, Olsen I, Tronstad L. Observation of *Saccharomyces cerevisiae* in blood of patient undergoing root canal treatment. Int Endod J **1997**; 30:313–7.
52. Olver WJ, James SA, Lennard A, et al. Nosocomial transmission of *Saccharomyces cerevisiae* in bone marrow transplant patients. J Hosp Infect **2002**; 52:268–72.
53. Dougherty SH, Simmons RL. Postoperative peritonitis caused by *Saccharomyces cerevisiae*. Arch Surg **1982**; 117:248.
54. Doyle MG, Pickering LK, O'Brien N, Hoots K, Benson JE. *Saccharomyces cerevisiae* infection in a patient with acquired immunodeficiency syndrome. Pediatr Infect Dis J **1990**; 9:850–1.
55. Eng RH, Drehmel R, Smith SM, Goldstein EJ. *Saccharomyces cerevisiae* infections in man. Sabouraudia **1984**; 22:403–7.
56. Chertow GM, Marcantonio ER, Wells RG. *Saccharomyces cerevisiae* empyema in a patient with esophago-pleural fistula complicating variceal sclerotherapy. Chest **1991**; 99:1518–9.
57. Hennequin C, Thierry A, Richard GF, et al. Microsatellite typing as a new tool for identification of *Saccharomyces cerevisiae* strains. J Clin Microbiol **2001**; 39:551–9.
58. McFarland LV. *Saccharomyces boulardii* is not *Saccharomyces cerevisiae*. Clin Infect Dis **1996**; 22:200–1.
59. Salonen JH, Richardson MD, Gallacher K, et al. Fungal colonization of haematological patients receiving cytotoxic chemotherapy: emergence of azole-resistant *Saccharomyces cerevisiae*. J Hosp Infect **2000**; 45:293–301.
60. Zerva L, Hollis RJ, Pfaller MA. In vitro susceptibility testing and DNA typing of *Saccharomyces cerevisiae* clinical isolates. J Clin Microbiol **1996**; 34:3031–4.
61. Clemons KV, McCusker JH, Davis RW, Stevens DA. Comparative pathogenesis of clinical and nonclinical isolates of *Saccharomyces cerevisiae*. J Infect Dis **1994**; 169:859–67.
62. Clemons KV, Hanson LC, Stevens DA. Colony phenotype switching in clinical and non-clinical isolates of *Saccharomyces cerevisiae*. J Med Vet Mycol **1996**; 34:259–64.
63. Mydlik M, Tkacova E, Szovenyiova K, Mizla P, Derzsiova K. *Saccharomyces cerevisiae* peritonitis complicating CAPD. Perit Dial Int **1996**; 16:188.
64. Snyder S. Peritonitis due to *Saccharomyces cerevisiae* in a patient on CAPD. Perit Dial Int **1992**; 12:77–8.
65. Posteraro B, Sanguinetti M, D'Amore G, Masucci L, Morace G, Fadda G. Molecular and epidemiological characterization of vaginal *Saccharomyces cerevisiae* isolates. J Clin Microbiol **1999**; 37:2230–5.
66. McCullough MJ, Clemons KV, Farina C, McCusker JH, Stevens DA. Epidemiological investigation of vaginal *Saccharomyces cerevisiae* isolates by a genotypic method. J Clin Microbiol **1998**; 36:557–62.
67. Garcia-Martos P, Hernandez JM, Mira J, Galan F, Marin P. *Saccharo-*

myces cerevisiae vaginitis [in Spanish]. Enferm Infecc Microbiol Clin **1996**; 14:453–4.

68. Nyirjesy P, Vazquez JA, Ufberg DD, Sobel JD, Boikov DA, Buckley HR. *Saccharomyces cerevisiae* vaginitis: transmission from yeast used in baking. Obstet Gynecol **1995**; 86:326–9.
69. Konecny P, Drummond FM, Tish KN, Tapsall JW. *Saccharomyces cerevisiae* oesophagitis in an HIV-infected patient. Int J STD AIDS **1999**; 10:821–2.
70. van Doorn HC, Coelingh Bennink F. Vaginal infection caused by *Saccharomyces cerevisiae* [in Dutch]. Ned Tijdschr Geneeskd **1995**; 139: 1093–5.
71. Senneville E, Ajana F, Gerard Y, et al. Bilateral ureteral obstruction due to *Saccharomyces cerevisiae* fungus balls. Clin Infect Dis **1996**; 23:636–7.
72. Almanza L, Debien B, Fontaine B, Brinquin L. Four hours for a record, or a severe fuminating cellulitis: can *Saccharomyces cerevisiae* be the causal agent [in French]? Ann Fr Anesth Reanim **1998**; 17:130–2.
73. Bernstein CN, Orr K, Blanchard JF, Sargent M, Workman D. Development of an assay for antibodies to *Saccharomyces cerevisiae:* easy, cheap and specific for Crohn's disease. Can J Gastroenterol **2001**; 15: 499–504.
74. Darroch CJ, Barnes RM, Dawson J. Circulating antibodies to *Saccharomyces cerevisiae* (bakers'/brewers' yeast) in gastrointestinal disease. J Clin Pathol **1999**; 52:47–53.
75. Candelli M, Papa A, Nista EC, et al. Antibodies to *Saccharomyces cerevisiae:* are they useful in clinical practice? Hepatogastroenterology **2003**; 50:718–20.
76. Main J, McKenzie H, Yeaman GR, et al. Antibody to *Saccharomyces cerevisiae* (bakers' yeast) in Crohn's disease. BMJ **1988**; 297:1105–6.
77. Belchi-Hernandez J, Mora-Gonzalez A, Iniesta-Perez J. Baker's asthma caused by *Saccharomyces cerevisiae* in dry powder form. J Allergy Clin Immunol **1996**; 97:131–4.
78. Candelli M, Nista EC, Nestola M, et al. *Saccharomyces cerevisiae*–associated diarrhea in an immunocompetent patient with ulcerative colitis. J Clin Gastroenterol **2003**; 36:39–40.
79. Kontoyiannis DP. Fluconazole inhibits pseudohyphal growth in *Saccharomyces cerevisiae*. Chemotherapy **2000**; 46:100–3.
80. Sobel JD, Vazquez J, Lynch M, Meriwether C, Zervos MJ. Vaginitis due to *Saccharomyces cerevisiae:* epidemiology, clinical aspects, and therapy. Clin Infect Dis **1993**; 16:93–9.
81. Barchiesi F, Arzeni D, Fothergill AW, et al. In vitro activities of the new antifungal triazole SCH 56592 against common and emerging yeast pathogens. Antimicrob Agents Chemother **2000**; 44:226–9.
82. Canton E, Peman J, Orero A, et al. In vitro activity of posaconazole against yeasts isolated in blood cultures [in Spanish]. Rev Esp Quimioter **2002**; 15:335–40.
83. Swinne D, Watelle M, Van der Flaes M, Nolard N. In vitro activities of voriconazole (UK-109, 496), fluconazole, itraconazole and amphotericin B against 132 non-albicans bloodstream yeast isolates (CANARI study). Mycoses **2004**; 47:177–83.
84. Espinel-Ingroff A. In vitro activity of the new triazole voriconazole (UK-109,496) against opportunistic filamentous and dimorphic fungi and common and emerging yeast pathogens. J Clin Microbiol **1998**; 36:198–202.
85. Pfaller MA, Messer S, Jones RN. Activity of a new triazole, Sch 56592, compared with those of four other antifungal agents tested against clinical isolates of *Candida* spp. and *Saccharomyces cerevisiae*. Antimicrob Agents Chemother **1997**; 41:233–5.

Med Sci Monit, 2001; 7(1): 121-124

Skin prick test response to enzyme enolase of the baker's yeast (Saccharomyces cerevisiae) in diagnosis of respiratory allergy

Marita Nittner-Marszalska[1], Irena Wójcicka-Kustrzeba[2], Ewa Bogacka[1], Janusz Patkowski[1], Rafał Dobek[1]

[1] Department of Internal Medicine and Allergology, Medical University, Wrocław, Poland
[2] Department of Biochemistry, Medical University, Wrocław, Poland

key words: fungal allergy, Saccharomyces cerevisiae enolase, baker's yeast enolase

SUMMARY

Background: *The aim of the study is to prove that Saccharomyces cerevisiae enolase, the major allergen of the baker's yeast, induces allergic immediate response in patients with inhalant allergy sensitized to Candida albicans extract.*

Material and methods: *The study was performed in three groups of patients: I. 20 atopic patients with respiratory allergy sensitized to Candida albicans and inhalant allergens (mite, feather, pollens) II. 30 patients with respiratory allergy, positive skin tests to inhalant allergens but negative skin tests to Candida albicans and other fungi; III. 20 nonatopic, healthy individuals. Skin prick test of purified enolase from Saccharomyces cerevisiae (bakers yeast) at concentration 1 and 10 mg/ml was performed in all groups. The results were documented planimetrically.*

Results: *95% of patients sensitized to Candida albicans extract showed positive skin reactions to Saccharomyces cerevisiae enolase, 10% of patients of group II and none of the patients of the control group had positive skin responses to enolase. The mean wheal size (mm^2) in skin prick test to Candida albicans, Saccharomyces cerevisiae enolase at concentration 10 mg/ml was $x=15.17\pm11.08$, 15.76 ± 19.67 and at concentration 1 mg/ml 10.02 ± 10.49, respectively.*

Conclusions: *1. Saccharomyces cereviasiae enolase induces an immediate allergic reaction in skin in subjects with respiratory allergy and positive skin prick test results to Candida albicans and other fungi. 2. Enolase can be an important allergenic component of the Candida albicans extract.*

BACKGROUND

Among numerous factors causing allergic disorders, fungi are considered to play an important role. Microfungi unquestionably are an important allergen source, and a principal cause of mould allergy. High concentration of spores in ambient air and their diameter allowing to penetrate distal airways facilitate fungal respiratory allergy.

Sensitization to some fungal species is more prevalent than to other species. The significance of fungi (Cladosporum herbarum, Alternaria alternata, Penicillum notatum, and Aspergillus fumigatus) in pathophysiology of bronchial asthma and allergic rhinitis is well recognized. Skin prick tests to their extracts are positive in 5–16% of the whole population, whereas the intracutaneous test, which is regarded to be less specific and more sensitive than prick test, is positive in as high a portion as 24% of the population [1]. At least 12% of allergic subjects are sensitized to Alternaria alternata, butt less than 7% may react to Cladosporum herbarum [1–3].

Received: 2000.07.28
Accepted: 2000.11.03

Correspondence address: Marita Nittner-Marszalska MD, Department of Internal Medicine and Allergology, Medical University, ul. Traugutta 57, Wrocław, Poland, e-mail: marmarsz@uni.wroc.pl

Candida albicans coexists with man as a harmless commensal in the digestive system and vaginal tract and less frequently as an opportunistic pathogen but its role as allergen is disputed. Several publications have suggested that Candida albicans is allergenic [4–7].

Other fungal species such as Pityrosporum ovale and Saccharomyces cerevisiae do not induce respiratory allergy but are an important pathogenic factor in urticaria and atopic dermatitis [7,8].

It was also observed that fungal allergy usually coexisted with a sensitization to other extrinsic aeroallergens.

A number of studies have been carried out on fungal allergens until now. The significance of numerous allergens including proteins (enolase and acid protease) and polysaccharides (mannan) has been reported [8–10]. A cross-allergenicity between some fungal species has been also well documented. It seems that one of the antigens responsible for this phenomenon could be a glycolytic enzyme enolase [10,12]. This enzyme has been proved to be a major allergen of Saccharomyces cerevisiae and Candida albicans, and one of allergens in extracts of Cladosporium herbarum and Alternaria alternata.

The aim of our study was to assess if enzyme enolase, isolated from Saccharomyces cerevisiae, is able to induce an immediate allergic reaction in skin prick test in subjects with respiratory allergy/skin allergy and positive skin prick test results to Candida albicans, and whether it can be responsible for the cross-reactivity between the fungal species.

MATERIAL AND METHODS

Subjects

The patients who entered the study were divided into three groups. The group I consisted of 20 atopic subjects, 12 male and 8 female, age: 24.1±9.2 (mean±SD), suffering from allergic rhinitis and/or bronchial asthma. They had a positive skin prick test to crude extract of Candida albicans. They also had positive skin prick test responses to some of the common extrinsic allergens: Dermatophagoides pteronyssimus, Dermatophagoides farinae, feather, dog, cat, grasses. Additionally, they had been tested to three fungal species Alternaria alternata, Cladosporum herbarum, Penicillum notatum: 11/20 showed positive reaction to Alternaria alternata, 9/20 to Cladosporum herbarum and 6/20 to Penicillum notatum. Group II consisted of 30 patients, 14 male and 16 female, age: 32.2±10.3, with symptoms of respiratory allergy confirmed by positive skin prick test to inhaled allergen extracts and negative skin tests to extracts of tested fungi. All of the patients were free of chest infections, had stable bronchial asthma and received no antihistamine or systemic corticosteroids medication during the time of the study. Group III (control) consisted of 20 subjects, 8 male and 12 female, age: 20.9±3.1, without any symptoms of respiratory allergy, and with negative skin prick test to inhaled allergens and fungal extracts. Before skin testing, a full explanation of the procedure had been given to all the participants and their consent was obtained.

Study design

Skin tests

In all groups skin prick tests to panel inhalant allergens, moulds, Candida albicans and enolase in concentrations 1 mg/ml and 10 mg/ml (Sigma cat. no. E 6126) were carried out according to Nordic recommendations [11]. The patients' skin was wiped with 70 percent ethanol and allowed to dry, after which the sites for testing were marked with a pen. Extracts for testing were supplied as 1:10 glycerinated solutions. One drop of each test extract was applied in duplicate on the healthy skin using a rubber bulb dropper, after which the skin was punctured through the drop with a sterile skin test lancet. To help avoid carryover of allergen from one test site to another, a separate needle was employed for each test extract. The needle punctures were deep enough to penetrate the epidermis but not to draw blood. After the puncture was made, the drop of allergen was wiped away after the time of two minutes. The results were measured after 20 minutes with a weal diameter exceeding 3 mm and more than 50% histamine test results regarded as positive. The positive test results were patterned on a transparent film to obtain a planimetric evaluation. The weal areas were compared and the final results were expressed in sq. mm.

Solution of histamine dihydrochloride 1 mg/ml was used as a positive control and saline-glycerol solution as a negative control. Positive and negative control tests were placed whenever a patient underwent skin testing. The negative control test

may aid in identifying patients who reacted non-specifically or exhibited dermographism to the minor skin trauma associated with testing. The positive control was done to prove if the skin reactivity was not diminished, for example by medications such as antihistamine preparations or psychotropic drugs.

All skin prick tests were performed on the volar aspect of the forearm with a distance of 5 cm between points.

All the allergens were fresh and used for the study only. Inhaled allergen extracts were manufactured by Allergopharma (Allergopharma Hamburg Germany).

RESULTS

All the study patients had negative results of the negative control test and positive results with histamine.

Out of the 20 atopic patients with respiratory allergy, whose skin tests showed positive responses to C. albicans and C. herbarum or/and A. alternata, 75% (15/20) had positive skin responses to enolase Saccharomyces cerevisiae at concentration of 1 mg/ml and 95% (19/20) positive reaction for enolase at concentration of 10 mg/ml. The mean weal areas of skin test with C. albicans was 15.17 ± 11.08 mm^2 while the mean weal area of skin test with enolase at concentration of 10 mg/ml was 15.76 ± 19.67 mm^2. Attention should be paid to the fact that skin prick areas to Candida albicans and Saccharomyces cerevisiae enolase have been very similar.

Among patients from group II with signs of atopy but without fungal sensitization (all skin tests with fungal allergens were negative) we have found highly positive skin prick test to enolase in three cases i.e. in 10% (3/30). In these patients the mean wheal area of skin test with enolase was 12.3 ± 10.09 mm^2.

We have not observed any positive skin tests to enolase in control group subjects at either concentration.

DISCUSSION

In the study group of atopic patients suffering from respiratory diseases and sensitive to Candida albicans, we have shown that 95% of the individuals manifested positive skin test results with baker's yeast enolase. Moreover, the results were very similar to the ones obtained in skin test with Candida albicans. Positive skin test to Saccharomyces cerevisiae enolase does not necessarily indicate a sensitization to this yeast, but may be a sign of an allergy to Candida albicans or even a different fungal species, which probably results from cross-reactivity among different fungal species.

Saccharomyces cerevisiae, the baker's yeast, is commonly used in foods. It may be also responsible for symptoms of chronic urticaria as well as atopic dermatitis. Positive skin prick tests to Saccharomyces cerevisiae and/or Pityrosporum ovale and /or Candida albicans are seen in as high a portion as 70-94% of patients with atopic dermatitis [8]. Some of the subjects with severe recurrent type of this disease synthesize also specific IgE antibodies against these yeasts [8,9]. Saccharomyces cerevisiae, contrary to other species, is not responsible for respiratory allergy apart from its possible role in the pathophysiology of baker's asthma. Hence a likelihood of its allergic impact in the study group of patients suffering from allergic respiratory diseases is very low.

Studies on the allergenic compounds of yeasts have shown that one of the major yeast allergens seems to be 51kD protein – enzyme enolase [12]. As it was proved by Baldo and Backer, among 47 subjects with positive skin prick test to baker's yeast, 23 reacted positively to enolase at concentration 10 mg/ml. Specific IgE against Saccharomyces cereviasiae enolase was detected in 22 patients among 32 with specific IgE against yeast extract [9]. The other important Saccharomyces cerevisiae allergens are: mannan – a compound of the cellular wall and enzyme alcohol dehydrogenase. These allergens are also detected in extracts of other fungi i.e. Candida albicans, Alternaria alternata and Cladosporum herbarum and may be responsible for cross-reactivity among various yeasts species. In spite of some structural similarity (homologous aminoacid sequence) between different types of enolase, antigenic determinants are not necessarily identical. Various enolases may have, besides common, their own epitopes for IgE antibodies. It could explain the fact, described by Sovolainen, that Saccharomyces cerevisiae enolase was able only in part to block binding Candida albicans enolase in immunoblotting reaction [6].

Positive skin test results with Saccharomyces cerevisiae in atopic individuals suffering from atopic

respiratory diseases but having negative skin test results with Candida albicans, Alternaria alternata, Cladosporum herbarum, and Penicillum notatum can be also accounted for by the phenomenon of cross-reactivity among fungal species. The fact can be explained in twofold manner: the studied individuals are either sensitive to baker's yeast, which however is not supported by clinical observation, or they are sensitive to a fungal species cross-reactive with baker's yeast enolase, not included in the present study.

These patterns of cross-reactivity are considered as important factors in pollen allergy and food allergy. Cross reactivity is also significant in fungal allergy. If fungal allergy is to be properly diagnosed and managed, the limits and extent of these patterns must be determined.

The importance of enolase in immunopathophysiology of respiratory allergy has not been entirely clarified yet. Further clinical investigations will be required to evaluate the role of this enzyme in the cross-reactivity with other yeast species.

The study suggests diagnostic usefulness of a screening test with Saccharomyces cerevisiae enolase in the diagnosis of individuals suspected of fungal allergy.

REFERENCES:

1. *Salvaggio J, Aukrust L: Mold induced asthma. J Allergy Clin Immunol, 1981; 68: 327-46*
2. *Al-Doory Y, Domson JF: Mould Allergy. Lea and Febiger, Philadelphia 1984*
3. *Jelks ML, Solomon R: Airborne allergens. In: Kaplan AP (ed.): Allergy. Churchill Livingstone 1985*
4. *Gumowski P, Lech B, Chaves I, Girard JP: Chronic asthma and rhinitis due to Candida albicans, Epidermophyton and Trichophyton. Ann Allergy, 1987; 59: 48-51*
5. *Akiyama K, Yui Y, Shida T, Miyamoto T: Relationship between the results of skin, conjunctival and bronchial tests and RAST with Candida albicans in patients with asthma. Clin Allergy 1981; 11: 343-51*
6. *Savolainen J, Viander M, Koivikko A: IgE, IgA and IgG antibody responses to carbohydrate and protein antigens of Candida albicans in asthmatic children. Allergy, 1990; 45: 54-63*
7. *Savolainen J, Lammintausta K, Kalimo K, Viander M: Candida albicans and atopic dermatitis. Clin Exp Allergy, 1993; 23: 332-9*
8. *Lintu P, Savolainen J, Kalimo K: IgE antibodies to protein and mannan antigens of Pityrosporum ovale in atopic dermatitis patients. Clin Exp Allergy, 1997; 27: 87-95*
9. *Baldo BA, Baker RS: Inhalant allergies to fungi: reactions to bakers yeast and identification of bakers yeast enolase as an important allergen. Int Arch Appl Immunol, 1988; 86: 201-208*
10. *Ishiguro A, Homma M, Torii M, Tanaka K: Identification of Candida albicans antigens reactive with immunoglobulin E antibody of human sera. Infection and Immunity, 1992; 1550-1557*
11. *Position statement. Allergen skin testing. J Allergy Clin Immunol, 1993; 92: 636-637*
12. *Franklyn KM, Warmington JR, Ott AK, Ashman RB: An immunodominant antigen of Candida albicans shows homology to the enzyme enolase. Immunol Cell Biol, 1990; 68: 173-178*

JOURNAL OF ASTHMA
Vol. 41, No. 2, pp. 223–228, 2004

ORIGINAL ARTICLE

Allergic Bronchopulmonary Fungal Disease Caused by *Saccharomyces cerevisiae*

Haruhiko Ogawa, M.D.,[1,*] Masaki Fujimura, M.D.,[2] and Yohei Tofuku, M.D.[1]

[1]Division of Internal Medicine, Ishikawa-ken Saiseikai Kanazawa Hospital, Kanazawa, Japan
[2]Respiratory Medicine, Cellular Transplantation Biology, Kanazawa Graduate University School of Medicine, Kanazawa, Japan

ABSTRACT

We describe a patient who presented with dry cough, low-grade fever, and focal patchy shadow of pulmonary infiltrates. Remarkably, the prospective etiological agent, *Saccharomyces cerevisiae* was purely and repeatedly cultured from her sputum. Allergic bronchopulmonary mycosis (ABPM) was diagnosed based on clinical, serological, and pathological criteria. Although the patient described here satisfied only three of the criteria, the conclusion that the allergic bronchopulmonary disease in our case was induced by *S. cerevisiae* was made based on the following evidence: 1) *S. cerevisiae* was repeatedly isolated from the patient's sputum, 2) anti-*S. cerevisiae* antibody was detected in her serum, and 3) bronchoprovocation test to *S. cerevisiae* antigen was positive. We present here a case of allergic bronchopulmonary fungal disease caused by *S. cerevisiae* antigen.

Key Words: Allergic bronchopulmonary fungal disease; *Saccharomyces cerevisiae*; Environmental survey; Bronchoprovocation test.

A number of fungal species may cause respiratory disorders, including allergic bronchopulmonary aspergillosis (1), bronchial asthma (2), eosinophilic pneumonia (3–5), eosinophilic bronchitis (6,7), and atopic cough (8–11). In some patients with allergic pulmonary disorders, the causative substrates can be successfully identified: drugs (12–14), parasitic and fungal infection (15,16), and allergic bronchopulmonary mycosis (ABPM) (17–19). We previously reported the importance of repeated sputum culture and environmental survey (5–8) for investigating the etiological agent in allergic pulmonary diseases. In the present study,

*Correspondence: Haruhiko Ogawa, M.D., Division of Pulmonary Medicine, Ishikawa-ken Saiseikai Kanazawa Hospital, Ni-13-6 Akatsuchi-machi, Kanazawa 920-0353, Japan; Fax: +81-76-266-1070; E-mail: saiseikh@po3.nsknet.or.jp.

DOI: 10.1081/JAS-120026080
0277-0903 (Print); 1532-4303 (Online)

we investigated a case of allergic bronchopulmonary fungal disease. Because *Saccharomyces cerevisiae* was purely and repeatedly cultured from the sputum of the patient, we investigated whether the fungus was an etiological agent.

CASE REPORT

A 25-year-old female was admitted to our hospital on January 14, 2000 because of dry cough, low-grade fever, and focal patchy shadow of pulmonary infiltrates. Further questioning revealed that the patient had been indeed healthy without a history of atopic diseases until the onset of the present illness. She was an office worker and lived with her family in an apartment house that was built 20 years ago and had scant direct sunlight. She was a never smoker.

Physical examination revealed the following: temperature 37.2° C; blood pressure 106/66 mmHg; heart rate 82 beats/min. The conjunctivae were not anemic or icteric. Cardiac examination was entirely within normal limits. Auscultation of the lungs revealed late inspiratory fine crackles on the right lung field without wheezes or rhonchi. Laboratory studies revealed that the white blood cell count (WBC) was 7200 μL with a differential of 90.4% segmented neutrophils, 7.2% lymphocytes, 1.5% monocytes, and 0.3% eosinophils. The hemoglobin level was 13.0 g/dl with a hematocrit value of 40.1%. The erythrocyte sedimentation rate was 20 mm/hr. The C-reactive protein was 5.6 mg/dl and the IgE level was 230 U/mL. Specific IgE antibodies to six common aeroallergens including *Aspergillus fumigatus* were negative. The following laboratory findings were normal or negative: urinalysis, stools for ova and parasites, serum electrolytes, total protein, albumin, and bacterial and mycobacterial cultures of sputum. Chest radiographs taken upon admission (Figure 1) showed consolidation on the right lung field. Chest CT scan taken upon admission (Figure 2) showed a cylindrical shadow surrounded by small granular shadows in the right S^5.

Pulmonary function test using the Collins DS system, which was performed according to the standards of the American Thoracic Society, (20) revealed a forced vital capacity (FVC) of 3.34 L (108.1% of predicted value), forced expiratory volume in 1 second (FEV_1) of 3.17 L (101.0% of predicted value), and FEV_1/FVC ratio of 94.9%.

Differential cell analysis of bronchoalveolar lavage fluid (BALF) revealed 59% alveolar macrophages, 34% lymphocytes, 6% neutrophils, 1% eosinophils, and an increased absolute total cell count of 1.7×10^6 cells/mL. Histological examination of transbronchial bronchial biopsy (TBBB) specimens obtained from the bifurcation of the right B^6 and the right lower bronchus (Figure 3) showed extensive eosinophil infiltration into the bronchial mucosa.

Because *S. cerevisiae* was purely and repeatedly cultured from the patient's sputum, it was suspected that eosinophilic bronchitis without asthma in this case might have been caused by *S. cerevisiae*.

For the preparation of fungal antigenic solutions (5–8), the fungus was cultured on medium (1% peptone, 2% glucose) containing 0.5% yeast extract for 20 days and dried by acetone. Skin tests with the fungal antigens were performed by intradermal injection of 0.02 mL of the antigen solution (1 mg/mL). The immediate (15 min) skin reaction was positive for *S. cerevisiae* but both the immediate and delayed (48 h) skin reactions were negative for *Penicillium janthinellum* as a control. The serum antifungus antibody titers measured by the Ouchterlony method were $\times 64$ for

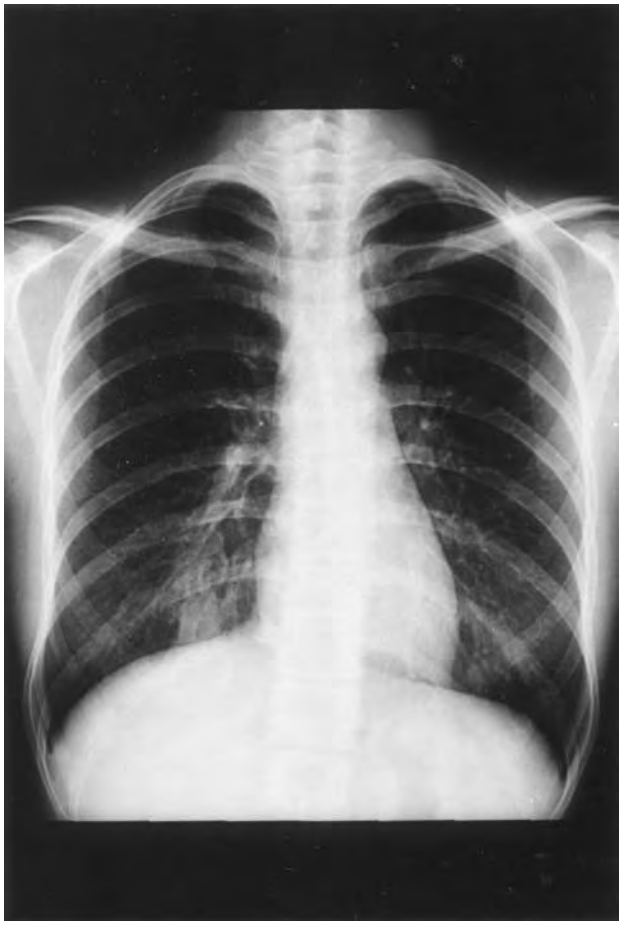


Figure 1. Chest radiographs on admission showed consolidation on the right lung field.

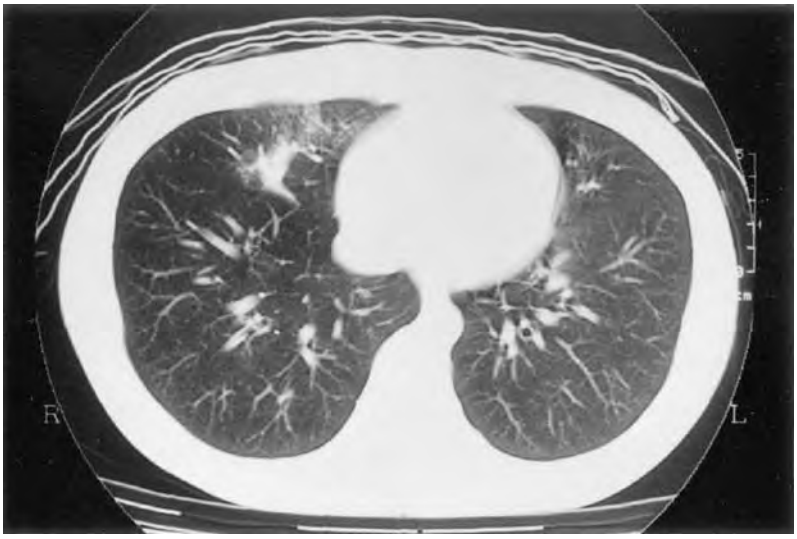


Figure 2. Chest CT scan on admission showed a cylindrical shadow surrounded by small granular shadows in the right S^5.

S. cerevisiae and ×8 for *P. janthinellum*, respectively (Table 1).

Seven days after admission, the patient's coughing gradually improved in response to beclomethasone dipropionate (BDP) inhalation therapy (600 Lg/day), and a chest radiograph showed further resolution. Although BDP therapy was discontinued upon discharge from our hospital, the patient's coughing had subsided on February 10 and *S. cerevisiae* had disappeared from her sputum.

On May 9, upon obtaining informed consent, the patient was readmitted for antigen bronchoprovocation testing (21) using *S. cerevisiae* and *P. janthinellum* antigens. A 2 mL dose of culture-filtrate antigen solution (1 mg/mL) was inhaled by tidal mouth breathing from a Devilbiss 646 nebulizer (Devilbiss Co, Somerset, PA) operated by compressed air at 5 L/min. The bronchoprovocation test using *P. janthinellum* antigen was negative but that using *S. cerevisiae* antigen was considered positive. The patient exhibited

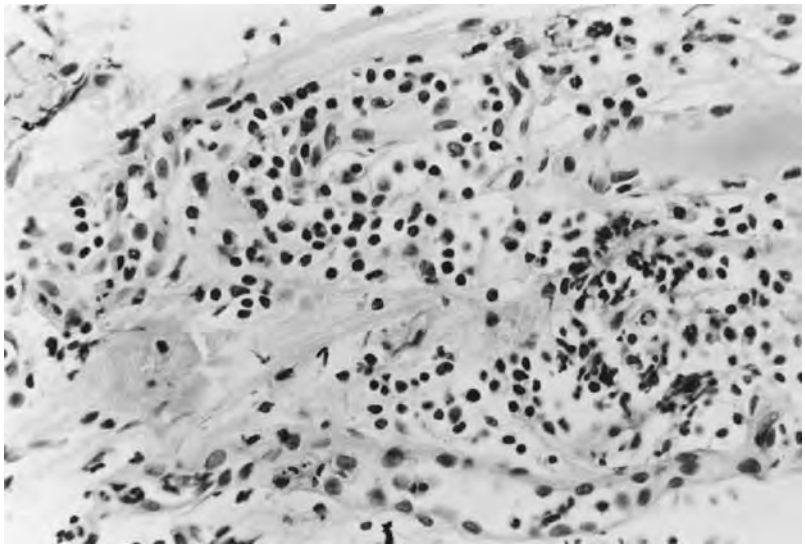


Figure 3. Histological examination of transbronchial bronchial biopsy (TBBB) specimens obtained from the bifurcation of the right B^6 and the right lower bronchus showed extensive eosinophil infiltration into the bronchial mucosa (H.E. stain ×50).

Table 1. Allergologic findings.

1) Skin reaction				
		Immediate	Late (6 hr)	Delayed (48 hr)
Saccharomyces cerevisiae		6×7/28×23	0×0/6×7	0×0/4×4
	(husband)	0×0/0×0	0×0/0×0	0×0/0×0
Penicillium janthinellum		4×4/4×4	0×0/0×0	0×0/0×0
	(husband)	0×0/0×0	0×0/0×0	0×0/0×0
2) Precipitating antibody				
Saccharomyces cerevisiae			×64	
	(husband)		×4	
Penicillium janthinellum			×8	
	(husbnad)		×4	

a coughing attack, a high fever (38.2° C), and a ticklish throat within 15 min, and the serum C-reactive protein (CRP) (from 0.4 to 1.6 mg/dl) and sputum eosinophils (from 40% to 70%) increased on the day after the provocation with *S. cerevisiae* antigen (Table 2). These symptoms disappeared within 3 days after the challenge, and the eosinophil levels in her sputum gradually decreased to 6% on June 19.

Table 2. Clinical and laboratory findings in the inhalation challenge test.

Penicillium janthinellum	Before	15 min	6 hr	48 hr
Symptoms	(−)	(−)	(−)	(−)
B.T.(°C)	36.7	36.6	36.6	36.4
Labolatory findings				
CRP(mg/dl)	0			0
WBC(/μL)	4900			5100
Eosinophil (%)	9.1			7.5
Cough threshold to capsaicin (μM)	15.6			31.3
Chest X-ray	Normal			No change
Saccharomyces cerevisiae				
	Before	15 min	6 hr	48 hr
Symptoms	(−)	*chill. cough*	(−)	(−)
B.T.(°C)	36.6	38.2	36.9	36.8
Laboratory findings				
CRP(mg/dl)	0.4			1.6
WBC(/μL)	5900			6400
Eosinophil(%)	4.1			3.4
Eosinophil in sputum(%)	40			70
Pulmonary function test				
FVC_1	3.26			3.34
$FEV_{1.0}$(l)	3.11			3.13
% DLco (%)	82.4			79.7
Cough threshold to capsaicin (μM)	31.3			31.3
Chest X-ray	Normal			No change

DISCUSSION

Saccharomyces cerevisiae (22), also known as brewer's or baker's yeast, is an ascomycetous yeast that is used in the production of baked goods, beer, and wine, and is occasionally found in health foods. Rarely, *Saccharomyces* has been reported to cause disease in humans (23). Reports of invasive infection by *S. cerevisiae* are few but include vaginitis (24,25), endocarditis, and pneumonia treated with prolonged antimycotic drug therapy. However, to our knowledge, allergic pneumonitis caused by *S. cerevisiae* has not been previously reported. We present a case of allergic bronchopulmonary fungal disease caused by *S. cerevisiae* antigen.

In the present case, *S. cerevisiae* was morphologically identified and purely and repeatedly cultured from the patient's sputum. The immediate type skin reaction was positive for *S. cerevisiae* and a high titer of anti-*S. cerevisiae* antibody was detected in the patient's serum. Furthermore, an inhalation bronchoprovocation test (21) using *S. cerevisiae* antigen was positive. Allergic bronchopulmonary mycosis is usually diagnosed by clinical and serological criteria, and detection of the fungus is not necessarily important for its diagnosis. The following clinical symptoms are used: 1) asthma, 2) a history of infiltrates on chest X-ray, 3) peripheral blood eosinophilia, 4) elevated total serum IgE, 5) immediate cutaneous reactivity to the particular fungus in question, 6) elevated specific IgE and IgG to the fungal antigen, and 8) presence or absence of central bronchiectasis (26). Although the

patient described here satisfied only three criteria—eosinophilic bronchitis without asthma, intermittent pulmonary infiltrates, and precipitins against fungus, the conclusion that the allergic bronchopulmonary disease in our case was induced by *S. cerevisiae* was made based on the following evidence: 1) *S. cerevisiae* was repeatedly isolated from the patient's sputum, 2) anti-*S. cerevisiae* antibody was detected in her serum, and 3) bronchoprovocation test using *S. cerevisiae* antigen was positive.

We previously proposed that beclomethasone dipropionate (BDP) inhalation therapy is effective for treating eosinophilic tracheobronchitis with airway cough hypersensitivity [diagnostic label ''atopic cough'' (8–11)]. Therefore, we applied the same therapy to this case of eosinophilic bronchitis. The patient's symptoms and patchy shadows on her chest X-ray gradually disappeared and *S. cerevisiae* cultured from her sputum diminished with the BDP inhalation therapy without administration of any antifungal agents. This therapy for eosinophilic bronchitis may repair the damaged mucocilliary transport, which would exclude the *S. cerevisiae* that had colonized the airway tract. As a result, various serological criteria for ABPM may remain within normal limits. Performing the sputum culture repeatedly in combination with routine serological testing to investigate the etiological agent in allergic bronchopulmonary fungal disease seems a useful approach.

ABBREVIATIONS

ABPM	allergic bronchopulmonary mycosis
S. cerevisiae	*Saccharomyces cerevisiae*
FVC	forced vital capacity
FEV_1	forced expiratory volume in 1 second
BALF	bronchoalveolar lavage fluid
TBBB	transbronchial bronchial biopsy
P. janthinellum	Penicillium janthinellum
BDP	beclomethasone dipropionate

ACKNOWLEDGMENT

The authors wish to thank Dr. Masakatsu Seo (Seo Laboratory) for his identification of the fungus species.

REFERENCES

1. Ricker DH, Taylor SR, Gartner JC Jr, Kurland G. Fatal pulmonary aspergillosis presenting as acute eosinophilic pneumonia in a previously healthy child. Chest 1991; 100:875–877.
2. Licorish K, Novey HS, Kozak P, Fairshter RD, Wilson AF. Role of ***Alternaria*** and ***Penicillium*** spores in the pathogenesis of asthma. J Allergy Clin Immunol 1985; 76:819–825.
3. Liebow AA, Carrington CB. The eosinophilic pneumonias. Medicine 1969; 48:251–285.
4. Ogawa H, Fujimura M, Matsuda T, Nakamura H, Kumabashiri I, Kitagawa S. Transient wheeze; eosinophilic bronchobronchiolitis in acute eosinophilic pneumonia. Chest 1993; 104:493–496.
5. Ogawa H, Fujimura M, Amaike S, Matsumoto Y, Kitagawa M, Matsuda T. Eosinophilic pneumonia caused by *Alternaria alternata*. Allergy 1997; 52:1005–1008.
6. Ogawa H, Fujimura M, Myou S, Kitagawa M, Matsuda T. Eosinophilic tracheobronchitis with cough hypersensitivity caused by *Streptomyces albus* antigen. Allergol Intern 2000; 49:83–87.
7. Ogawa H, Fujimura M, Amaike S, Nishiura Y, Nakagawa-Yoshida K, Suga M, Ando M, Matsuda T. Seasonal chronic cough with sputum eosinophilia caused by *Trichosporon cutaneum* (*Trichosporon asahii*). Int Arch Allergy Immunol 1998; 116:162–165.
8. Ogawa H, Fujimura M, Matsumoto Y, Matsuda T. A case of atopic cough caused by *Pichia guilliermondii* successfully treated with anti-fungal therapy. J Jpn Respir Soc 1999; 37:209–213.
9. Fujimura M, Ogawa H, Yasui M, Matsuda T. Eosinophilic tracheobronchitis and airway cough hypersensitivity in chronic non-productive cough. Clin Exp Allergy 2000; 30:41–47.
10. Fujimura M, Kamio Y, Hashimoto T, Matsuda T. Cough receptor sensitivity and bronchial responsiveness in patients with only chronic nonproductive cough: in view of effect of bronchodilator therapy. J Asthma 1994; 31:463–472.
11. Fujimura M, Songur N, Kamio Y, Matsuda T. Detection of eosinophils in hypertonic saline-induced sputum in patients with chronic nonproductive cough. J Asthma 1997; 34:119–126.
12. Rosenow EC. The spectrum of drug-induced pulmonary diseases. Ann Intern Med 1972; 77:977–991.
13. Ogawa H, Kurashima K, Namura M, Kanaya H, Kawamura Y, Ohka T, Kurumaya H, Yasui M, Fujimura M, Matsuda T. Pulmonary infiltrates with eosinophils due to naproxan. Jpn J Med 1991; 30:32–34.
14. Ogawa H, Fujimura M, Heki U, Kitagawa M, Matsuda T. Eosinophilic bronchitis presenting

with only severe dry cough due to bucillamine. Respir Med 1995; 89:219–221.
15. Lombard CM, Tazelaar MD, Kransel DL. Pulmonary eosinophilia in coccidioidal infections. Chest 1987; 91:734–736.
16. Katzenstein AL, Askin FB. Surgical Pathology of Nonneoplastic Lung Disease. 2nd ed. Philadelphia: W.B. Saunders Co., 1990:182–196.
17. Mark AM, Paul AG, Roger A, John HT, Gary AN, Mary R, Roy P. Allergic bronchopulmonary mycosis caused by *Pseudallescheria boydii*. Am Rev Respir Dis 1993; 148:810–812.
18. Kenneth SB, Mary R, Roy P. Allergic bronchopulmonary mycosis caused by *Fusarium vasinfectum*. Am J Respir Crit Care Med 1995; 152:1379–1381.
19. Sunil KS, Steven RB, Ashley J, Mary R, Paul AG. Allergic bronchopulmonary mycosis to *Fusaarium vasinfectum* in a child. Ann Allergy Asthma Immun 1998; 80:377–380.
20. American Thoracic Society. Standardization of spirometry—1987 update. Am Rev Respir Dis 1987; 136:1285–1298.
21. Hendrick DJ, Marshall R, Faux JA, Krall JM. Positive ''alveolar'' responses to antigen inhalation provocation tests: their validity and recognition. Thorax 1980; 35:35.
22. Rippon JW. Characteristics of Fungi. Medical Mycology. 12th ed. Philadelphia: W.B. Saunders Co., 1982:117–139, , chapter 6.
23. Eng RHK, Drehmel R, Smith SM, Goldstein EJC. *Saccharomyces cerevisiae* infections in man. J Med Vet Mycol 1984; 22:403–407.
24. Aucott JN, Fayen J, Grossnicklas H, Morrissey A, Lederman M, Salata RA. Invasive Infection with *Saccharomyces cerevisiae*; report of three cases and review. Rev Infect Dis 1990; 12:406–411.
25. Sobel JD, Vazquez J, Lynch M, Meriwether C, Zervos MJ. Vaginitis due to *Saccharomyces cerevisiae*; epidemiology, clinical aspects, and therapy. Clin Infect Dis 1993; 16:93–99.
26. Rosenberg M, Patterson R, Mintzer R, Cooper BJ, Roberts M, Harris KE. Clinical and immunologic criteria for the diagnosis of allergic bronchopulmonary aspergillosis. Ann Intern Med 1977; 86: 405–414.

Marcel Dekker, Inc.
270 Madison Avenue, New York, New York 10016

Antonie van Leeuwenhoek **82:** 279–289, 2002.

Probiotics: an overview of beneficial effects

Arthur C. Ouwehand[1,*], Seppo Salminen[1] & Erika Isolauri[2]
[1]*Department of Biochemistry and Food Chemistry, University of Turku, FIN-20014 Turku, Finland;* [2]*Department of Paediatrics, Turku University Central Hospital, FIN-20500 Turku, Finland (*Author for correspondence; Tel.: +358-2-333-6894; Fax: +358-2-333-6860; E-mail: arthur.ouwehand@utu.fi)*

Key words: health effects, lactic acid bacteria, probiotics

Abstract

Food products fermented by lactic acid bacteria have long been used for their proposed health promoting properties. In recent years, selected probiotic strains have been thoroughly investigated for specific health effects. Properties like relief of lactose intolerance symptoms and shortening of rotavirus diarrhoea are now widely accepted for selected probiotics. Some areas, such as the treatment and prevention of atopy hold great promise. However, many proposed health effects still need additional investigation. In particular the potential benefits for the healthy consumer, the main market for probiotic products, requires more attention. Also, the potential use of probiotics outside the gastrointestinal tract deserves to be explored further. Results from well conducted clinical studies will expand and increase the acceptance of probiotics for the treatment and prevention of selected diseases.

Abbreviations: IBD – Inflammatory bowel disease; CD – Crohn's disease; UC – Ulcerative colitis

Introduction

The development of probiotics during the past decade has signalled an important advance in the food industry transferring to towards the development of functional foods. The term probiotic, popularised by R. Fuller in 1989, was defined recently by an Expert Committee as 'Living micro-organisms which upon ingestion in certain numbers exert health benefits beyond inherent general nutrition' (Guarner & Schaafsma 1998). Such a definition does not require changes in intestinal microflora or so-called 'colonisation' or temporary colonisation of the human gastrointestinal tract as the probiotic organism can exert its effects locally or during transient passage through the gastrointestinal system. This definition, however, still sets the requirements that the micro-organisms must be alive, not pasteurised or otherwise inactivated. Although specific numbers are not mentioned in the definition, generally it is thought that at least 10^9 colony forming units per day need to be ingested. Health benefits must be scientifically established by clinical studies in humans performed by several independent research groups and published in peer-reviewed journals.

The definition may be changing little by little as especially Japanese scientists have shown that also inactivated probiotic micro-organisms or their cell structures may have beneficial effects on human health. This has led to new definitions of probiotics and may change the way we look at probiotics in the future (Lee et al. 1999; Salminen et al. 1999).

Micro-organisms used as probiotics

Microbes from many different genera are being used as probiotics (Table 1). The most commonly used strains are members of the heterogeneous group of lactic acid bacteria; lactobacilli, enterococci and bifidobacteria. In particular lactobacilli are generally used as probiotics. This may have historical reasons since Metchnikoff proposed that the lactobacilli present in yoghurt would have a health promoting effect. Also, the most common means of administration is still a fermented dairy product. However, other microbes and even yeasts have been developed as potential probiotics during recent years (Table 1).

The choice what microbe to use as a probiotic is determined by many different factors (Table 2).

Table 1. Microbes used as probiotics and their documented health benefits in human clinical trials

Genus	Species	Example strains	Health benefit	Reference
Lactobacillus	*acidophilus*	La5	Reduced antibiotic associated diarrhoea	Black et al. 1991
	casei	Shirota	Shortening of rotavirus diarrhoea	Sugita & Togawa 1994
			Reduced recurrence of superficial bladder cancer	Aso et al. 1995
			Immune modulation	Nagao et al. 2000
	crispatus			
	fermentum	KLD		
	johnsonii	La1	Improved oral vaccination	Link-Amster et al. 1994
			Reduced colonisation by *Helicobacter pylori*	Felley et al. 2001
	paracasei	F19		
	plantarum	299v	Relief of irritable bowel syndrome	Niedzielin et al. 2001
			Reduction of LDL-cholesterol	Bukowska et al. 1998
	reuteri	SD2112	Shortening of rotavirus diarrhoea	Shornikova et al. 1997
	rhamnosus	GG	Shortening of rotavirus diarrhoea	Guandalini et al. 2000
			Immune modulation	Kaila et al. 1992
			Relief of inflammatory bowel disease	Gupta et al. 2000
			Treatment and prevention of allergy	Kalliomäki et al. 2001b; Majamaa & Isolauri 1997
	salivarius	UCC118	Reduced symptoms of inflammatory bowel disease	Mattila-Sandholm et al. 1999
Bifidobacterium	*breve*		Reduced symptoms of irritable bowel disease	Brigidi et al. 2001
	longum	BB536		
	lactis	Bb12	Treatment of allergy	Isolauri et al. 2001
			Shortening of rotavirus diarrhoea	Saavedra et al. 1994
			Reduced incidence of travellers diarrhoea	Black et al. 1989
			Improved oral vaccination	Link-Amster et al. 1994
Propionibacterium	*freudenreichii*	JS		
Bacillus	*subtilis*			
	cereus	toyoi		
Escherichia	*coli*	Nissle 1917	Fewer relapses of inflammatory bowel disease	Malchow 1997
Enterococcus	*faecium*	SF68		
Saccharomyces	*cerevisiae*	*boulardii*	Fewer relapses of inflammatory bowel disease	Guslandi et al. 2000

Table 2. Main properties for probiotic bacteria

Property	Benefit
Resistance to pancreatic enzymes, acid and bile	Survival of passage through the intestinal tract
Adhesion to the intestinal mucosa	Immune modulation
	Pathogen exclusion
	Enhanced healing of damaged mucosa
	Prolonged transient colonisation (?)
Human origin	Species specific interactions with the host
Documented health effects	Proposed health effects are 'true'
Safe	No health risk to consumer
Good technological properties	Strain stability
	Production at large scale
	Oxygen tolerance

In order to survive passage through the gastrointestinal tract, resistance to low pH, bile and pancreatic enzymes are important. Acid and bile tolerance can be easily monitored and they are considered intrinsic properties of lactic acid bacteria. Thus, in fermented milks acid stability is already required during the fermentation. Adhesion to the intestinal mucosa is considered important for immune modulation (the intestine is the largest immune organ of the body), pathogen exclusion, enhanced healing of damaged mucosa and prolonged transient colonisation. To obtain reasonable assurance on adherence, the use of at least two different test systems is required to describe both mucus and epithelial adhesion which represent the early and late stages of adherence to the mucosa. Human origin is thought to be important for host specific interactions by the probiotic, although e.g. *S. cerevisiae* (*boulardii*) is not of human origin. The microbes administered should obviously be safe. This is, however, often not specifically assessed. Lactobacilli and bifidobacteria are simply considered safe based on their taxonomic position. Although this may seem improper, it is difficult to assess the safety of generally non-pathogenic species. In practice, the first human feeding trial will also be the first safety trial, although this is often not recognised as such. Finally, potential probiotics need to have good technological properties so that they can be cultured on large scale, have an acceptable shelf life and, in case of application in fermented products, contribute to a good taste.

Lactobacilli have often good resistance to the *in vivo* stresses, as described in the next section, and several strains have good technological properties. This may, in addition to the historical reasons, explain their frequent use as probiotics. Bifidobacteria are also commonly used, though less then lactobacilli. They are sensitive to oxygen and have more strict growth requirements. This makes them technologically more difficult to use. The other probiotic species are, with the exception of propionibacteria and enterococci, usually not used in fermented products but as dietary supplements, in capsules, powders, etc.

Gut mucosal barrier: first line in host defence

The gastrointestinal tract is a complex microenvironment where the cells of the largest lymphoid organ of the human body interface with a myriad of endogenous and exogenous stimuli. The intestinal mucosa provides protective host defence to the constant presence in the gut lumen of antigens from food and the normal microflora.

Protection against potentially harmful agents is ensured by a number of factors including saliva, gastric acid, peristalsis, mucus, intestinal proteolysis, intestinal flora, and epithelial cell membranes with the intercellular junctional complexes (Sanderson & Walker 1993). Together with the well-functioning immunological defence, these processes provide antigen exclusion in the gut. However, there are specialised antigen transport mechanisms in the villous epithelium (Ducroc et al. 1983). Antigens are absorbed across the epithelial layer by transcytosis, and here the main degradative pathway entails lysosomal processing of the antigen. This second line of defence, immune elimination, is directed towards removal of antigens that have penetrated the mucosa. A minor pathway allows the

transport of unprocessed antigens (Isolauri 1999; Heyman & Desjeux 2000). The immunological regulation takes place in several compartments: aggregations of lymphoid cells in follicles and the Peyerþs patches, distributed within the mucosa and in the intestinal epithelium, as well as in secretory sites (Brandtzaeg 1995). The intraepithelial T-lymphocytes have mainly a suppressor/cytotoxic phenotype, while the lamina propria cells show the helper/inducer phenotype. Peyerþs patches, crucial in determining the subsequent immune responses to the antigen, are covered by the M-cells. In general, antigen transport across this epithelium is characterised by rapid uptake and reduced degradation (Ducroc et al. 1983).

The lamina propria is also endowed with lymphocytes belonging to the B-cell lineage. IgA antibody production is abundant at mucosal surfaces, where secretory IgA is present in dimeric or polymeric form. Secretory IgA is relatively resistant to intra-luminal proteolysis and does not activate complement or inflammatory responses. There are differences between the upper and lower parts of the human gut-associated immune system in the isotype distribution of immunoglobulin-producing cells (Brandtzaeg 1995; Salminen et al. 1998). IgA1 immunocytes predominate in the small intestine while IgA2-producing cells are most frequent in the colon, the latter being more resistant to bacterial proteases. The secretory IgA antibodies in the gut are part of the common mucosal immune system including respiratory tract and lacrimal, salivary and mammary glands. Consequently, an immune response initiated in the gut-associated lymphoid tissue can affect immune responses at other mucosal surfaces.

The intestine's mucosal surface provides a defence barrier against antigens encountered by the enteric route. As a result of the barrier function, systemic hyporesponsiveness to antigens such as food proteins, oral tolerance, is a hallmark of the intestinal immune system. In this system also a balance is generated and maintained between the host and the normal resident microflora. In addition to antigen degradation and thereby participating in tolerance induction, intestinal colonisation acts as an important endogenous stimulus for the maturation of the gut-associated lymphoid tissue (Helgeland et al. 1996). So far, the human gut microflora is still an unexplored organ of host defence and its impact in health and disease may be stronger than currently known. As stated by MacDonald (2001): "It is likely that the normal flora also produces immunoregulatory molecules and it is not entirely unfeasible that the disease-free state of the gut in normal individuals is caused by the flora and not by sophisticated immunoregulatory circuits".

Health effects of probiotics

Probiotic therapy and modulation of the intestinal microflora

The original idea with probiotics has always been to change the composition of the normal intestinal microflora from a potentially harmful composition towards a microflora that would be beneficial for the host. In general this would mean a reduction in the number of, e.g. coliforms and clostridia and an increase in lactobacilli and/or bifidobacteria. Probiotics that survive gastrointestinal transit are likely to cause an increase in faecal levels of that particular genus, especially when initial levels were low. Due to competition for adhesion sites and nutrients, and possibly the production of antimicrobial substances, levels of certain less desirable genera can decrease. A concomitant increase in faecal levels of genera other than the probiotic consumed has also been observed for certain probiotics. E.g. consumption of *L. rhamnosus* GG has been observed to be associated with an increase in faecal bifidobacteria (Benno et al. 1996) and consumption of *L. salivarius* UCC118 caused an increase in faecal *Enterococcus* levels (Mattila-Sandholm et al. 1999).

It is obvious that avoiding colonisation by pathogens and reducing the risk for over growth of potential pathogenic bacteria is beneficial to the host. However, in some cases too much emphasis is placed on this change in microflora composition without considering the actual health benefit. A mere change in intestinal microflora composition is not a sufficient biomarker for a potential health benefit of a given probiotic strain. Moreover, for some health effects, like immune modulation, it may not be necessary to obtain a measurable modification of the intestinal microflora composition.

Immune modulation by probiotics

The demonstration that in the absence of the intestinal microflora antigen transport is increased indicates that the gut microflora is an important constituent in the intestines defence barrier. In affecting the development of gut-associated lymphoid tissue at an early age the gut microflora directs the regulation of systemic and local immune responsiveness, including hyporesponsiveness to antigens derived from micro-organisms and

food. Experimental animals lacking interleukin-10 or transforming growth factor-β generate a mucosal inflammatory response to the resident gut microflora (Groux et al. 1999). The role of the intestinal microflora in oral tolerance induction has been investigated in germ-free mice (Sudo et al. 1997). In contrast to control mice, germ-free animals were seen to maintain their tendency to a systemic immune response, for example production of IgE antibodies, upon oral antigen administration. Abrogation of oral tolerance was due to the absence of intestinal flora. The aberrant IgE response could be corrected by reconstitution of the microflora at the neonatal stage, but not at a later age. In human infants, colonisation has been associated with the maturation of humoral immune mechanisms, particularly of circulating IgA- and IgM-secreting cells (Grönlund et al. 2000), reflecting the dependency of the regulation of the mucosal immune response on the normal gut microflora.

In several gut-related inflammatory conditions the healthy host-microbe interaction is disturbed and inflammation is accompanied by imbalance in the intestinal microflora in such a way that an immune response may be induced by resident bacteria (Isolauri 1999). Normalisation of the properties of unbalanced indigenous microflora by specific strains of the healthy gut microflora constitutes the rationale in probiotic therapy. The success of probiotic therapy manifests itself in normalisation of the increased intestinal permeability and altered gut microecology, improvement of the intestine's immunological barrier functions and alleviation of the intestinal inflammatory response. The targets for probiotic therapy are identified as clinical conditions involving impaired mucosal barrier function, particularly infectious and inflammatory diseases (Isolauri 2001).

Probiotics and allergic disease

The prevalence of atopic diseases has been progressively increasing in Western societies. The hygiene hypothesis conceives the rapid increase in atopy to be related to reduced exposure to microbes at an early age and subsequent lower number of infections in early life (Strachan 1989). This is related to smaller family size, vaccinations, consumption of almost sterile food and over hygienic practices in Western societies, which may cause the infants immune system to develop an inflammatory response. The earliest and most massive source of such exposure is associated with the establishment of the gut microflora. Indeed, differences in the neonatal gut microecology were recently documented as being associated with the development of atopic diseases (Kalliomäki et al. 2001a).

The T helper (TH) 2 responder phenotype is associated with enhanced production of IgE antibodies against ubiquitous environmental antigens, eosinophilia, and consequently constitutes a hallmark of atopic diseases. Specific strains of the gut microflora have been shown to contribute to the generation of counter-regulatory TH1- and TH3-type immune responses (Isolauri et al. 2001). In addition, these contribute to the processing of food antigens in the gut and reduce their immunogenicity *in vitro* and *in vivo*, together with a potential to dampen inflammatory responses to these antigens (Sütas et al. 1996; Majamaa et al. 1997; Isolauri et al. 2000; Pessi et al. 2000a).

The regulatory role of probiotics in allergic disease was first emphasised in a demonstration of a suppressive effect on lymphocyte proliferation and interleukin-4 generation *in vitro* (Sütas et al. 1996). Subsequently, the immunoinflammatory responses to dietary antigens in allergic individuals were shown to be alleviated by probiotics, this being partly attributable to enhanced production of anti-inflammatory cytokines, e.g. interleukin-10 (Pessi et al. 2000b) and transforming growth factor-β (Haller et al. 2000), and partly due to control of allergic inflammation in the gut (Majamaa & Isolauri 1997). The mucosal dysfunction caused by inflammation, characterised by the altered rate, route and mode of antigen presentation, is stabilised by probiotics (Isolauri 2001). So far, clinical effects have been seen as a significant improvement in the course of atopic eczema in infants given probiotic-supplemented elimination diets (Majamaa & Isolauri 1997; Isolauri et al. 2000). The preventive potential of probiotics in atopic disease has recently been demonstrated in a double-blind, placebo-controlled study (Kalliomäki et al. 2001b). Probiotics administered pre- and postnatally for 6 months to children at high risk of atopic diseases succeeded in reducing the prevalence of atopic eczema to half as compared with that in infants receiving placebo.

Probiotics in diseases of the gut

Probiotics have traditionally been used to treat disease related to the gastrointestinal tract, although other diseases have also been suggested to be relieved by the use of probiotics.

Lactose intolerance

Lactose intolerance, or more correctly lactose maldigestion, is caused by a reduced production of β-galactosidase. This is a normal condition in all adult mammals, with the exception of people from north-west European decent, and should therefore not be considered a disease as such. In these subjects, consumption of lactose leads to an increased osmotic load in the small intestine with subsequent secretion of fluids which leads to loose stools (Launiala 1968). The origin of the abdominal pain that is associated with the consumption of lactose by lactose maldigesting subjects is not well understood though it does not appear to relate to the production of gasses from the fermentation of lactose by the intestinal microflora (Lasser et al. 1975). Fermented milk products have been observed to be tolerated well by lactose maldigesters as compared to milk. This can be explained by the presence of β-galactosidase in the bacteria fermenting the milk. Upon ingestion, the bacteria are lysed by bile in the small intestine, the enzyme is released and degrades lactose. In addition to this, the more viscous properties of fermented milks, compared to plain milk, gives them a longer gastro-caecal transit time, thus further aiding digestion of lactose (Vesa et al. 2000). This beneficial effect is usually more associated with products fermented with *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus*. To what extend probiotics contribute to relief of lactose intolerance symptoms is uncertain, some probiotics like, e.g. *L. rhamnosus* GG are not able to ferment lactose.

Acute gastro-enteritis

Acute gastro-enteritis may have bacterial or viral origin. Rotavirus is one of the most common causes of acute childhood diarrhoea in industrial countries (Claeson & Merson 1990). Rotavirus invades and replicate in the differentiated absorptive columnar cells of the small intestinal epithelium. This results in partial disruption of the intestinal mucosa with loss of microvilli, a decrease in the villus/crypt ratio and an increased intestinal permeability (Salim et al. 1990). Several studies have shown that selected probiotics, such as *L. rhamnosus* GG, *L. reuteri*, *L. casei* Shirota and *B. lactis* Bb12, can shorten the duration of rotavirus diarrhoea by approximately 1 day (Kaila et al. 1992; Saavedra et al. 1994; Sugita & Togawa 1994; Shornikova et al. 1997). Several mechanisms maybe behind this favourable out come. The production of rotavirus specific IgA has been observed to be enhanced in response to treatment with certain probiotics (Kaila et al. 1992), the permeability of the intestinal mucosa has been observed to be reduced (Isolauri et al. 1993) and the composition of the intestinal microflora normalised (Salminen et al. 1996).

Antibiotic associate diarrhoea (AAD) is mainly due to an overgrowth of *Clostridium dificille*. In particular, *Saccharomyces cerevisiae* (*boulardii*) has been observed to reduce the risk for AAD (Surawicz et al. 1989). The incidence of AAD was less than half or a third in the *S. cerevisiae* (*boulardii*) group compared to the control group. Also other probiotics like *Lactobacillus rhamnosus* GG, *L. acidophilus* and *Enterococcus faecium* SF68 have been observed to prevent or treat AAD (Gismondo et al. 1999).

Inflammatory bowel disease

Inflammatory bowel disease (IBD) is clinically characterised by two overlapping phenotypes, Crohn's disease (CD) and ulcerative colitis (UC), which predominantly affect the colon (UC and CD) and/or the distal small intestine (CD). The aetiology of the disease is not completely understood, but a genetic predisposition and the normal intestinal microflora are thought to play an important role. Modifying the composition and activity of the normal microflora may thus improve the disease. Indeed selected probiotics have been observed to reduce the number of relapses and prolong the period of remission. Interestingly, not only lactic acid bacteria, *L. salivarius* UCC118 and *L. rhamnosus* GG, but also *S. cerevisiae* (*boulardii*) and a strain of *E. coli* (Nissle) have been observed to be effective in alleviating the symptoms of IBD (Mattila-Sandholm et al. 1999; Gupta et al. 2000; Guslandi et al. 2000; Hamilton-Miller 2001).

Colorectal cancer

The aetiology of colorectal cancer is diverse and diet has clearly been indicated to be involved (Greenwald et al. 2001). Diets, especially high in meat and fat or low in fibre, have been observed to cause changes in the composition of the intestinal microflora, with increasing levels of *Bacteroides* and*Clostridium* and decreased levels of *Bifidobacterium* (Benno et al. 1991). This change in microflora composition is associated with an increase in faecal enzyme activity, β-glucuronidase, azoreductase, urease, nitroreductase and glycocholic acid reductase. These enzymes con-

vert procarcinogens into carcinogens and may thus contribute to an increased risk for colorectal cancer. The consumption of selected lactobacilli have been observed to reduce this faecal enzyme activity. Whether this also reduces the actual risk for colorectal cancer remains to be proven. However, most, but not all, epidemiological studies suggest that regular consumption of fermented dairy products are related to lower risk for certain types of cancer (Hirayama & Rafter 2000). Some positive effect of probiotic lactic acid bacteria on the risk for colorectal cancer can therefore be anticipated although definite proof remains to be presented.

Constipation

Constipation is a major digestive complaint among the elderly, in particular the institutionalised. Although also, otherwise healthy, adults and hospitalised subjects may experience constipation. Constipated subjects have been observed to have a modified faecal microflora with reduced levels of bifidobacteria, *Bacteroides* and, in particular, reduced levels of clostridia (Shimoyma et al. 1984). Probiotics have been suggested to relieve constipation (Goldin 1998; Lee et al. 1999). However, review of the literature does not substantiate this claim. This may relate to the causes of constipation; physical inactivity, low-fibre diets, insufficient liquid intake and some drugs. The altered microflora composition is more likely to be a consequence than the cause of constipation, correcting the microflora composition may therefore not be of help.

Benefits for healthy subjects

Determining the potential health effects of probiotics for healthy subjects is difficult although this is of major importance since probiotics are mainly marketed for healthy subjects. The health effects of probiotics on healthy subjects are likely to be limited to risk reduction. As mentioned above, consumption of fermented dairy products maybe related to a reduced risk for colorectal cancer. However, that evidence is rather circumstantial. More direct evidence suggests that, at least in children, long term consumption of probiotics in non-fermented milk may reduce the risk for infections, absence from day care due to illness and the use of antibiotics (Hatakka et al. 2001). This study indeed indicates that probiotics can also be of benefit to the healthy consumer. Probiotics are often marketed as 'boosting the immune system'. For healthy individuals this may not be the case, since the immune system is likely to be working optimally (Spanhaak et al. 1998). However, in combination with oral vaccination, improved antibody titres have been observed with probiotics (Link-Amster et al. 1994).

Developing future probiotic strains

Quality of probiotic strains

Probiotics are special ingredients that are used in both foods and pharmaceutical or special dietary applications. They have been selected to express strain specific properties which are important for their proposed health effects. Such characteristics should be retained through food and pharmaceutical processes and storage to be of benefit to the consumer.

The most important factor is to retain the strain characteristics and the purity of the preparation. It has been reported that especially dried probiotic preparations may have contaminants. This sets the requirements for hygienic preparation of the products and careful identification of the strains used. All commercial strains should be placed in an international type culture collection for future comparison of the properties and identity. Some probiotic preparations may also mislabel the strains they contain, using old or non-existing nomenclature.

Unlike pharmaceuticals or food chemicals such as additives, the quality criteria for probiotics are largely undefined. This is a key factor for health effects as long term transfer of probiotic lactic acid bacteria or bifidobacteria in food processing along with the storage may result in changes in their characteristics and health properties. To control these properties, criteria for assessing such changes should be included in functional food regulations. The criteria currently used for selecting new probiotics have been suggested as the optimal quality control measures to be used in industrial practise (Tuomola et al. 2001).

In recent studies the necessity of testing the stability of strain characteristics was established for model bacteria and common probiotics used in foods (Tuomola et al. 2000, 2001). Adherence properties can be considered as the main selection criterion for current probiotics and adherence is important for both local colonisation and immune modulation through contact with the gut associated lymphoid tissue. Adherence varies greatly among the current probiotic strains and

adherence characteristics can vary in two different *in vitro* models. Processing and gastric secretions also influence adhesion and they should be taken into consideration (Tuomola et al. 2000; Ouwehand et al. 2001).

Early reports have documented that adherence properties depend on culture conditions, the number of transfers in industrial scale fermentation and use of cryoprotectants in freeze-drying (Elo et al. 1991). Transfer of cultures in processing over a period of 3 years decreased adhesion and changing the culture medium could also result in diminished adhesion properties (Elo et al. 1991; Tuomola et al. 2001). We have also shown that specific probiotics isolated from a similar product from different countries show very different adhesion properties. Viability is crucial for lactic acid bacteria used as starter cultures or probiotics.

Viability may be important for health effects as currently most of the clinical evidence has been reported for viable strains and relatively few effects have been documented for non-viable strains (Ouwehand & Salminen 1998). There are recent reports on the viability of probiotic formulations in Britain and the United States and they demonstrate a lack of quality control in this respect. In the US, of 30 supplements tested 11 contained no viable bacteria and in Britain only six out of 13 formulations were satisfactory in terms of viability (Temmerman et al. 2001; Hamilton-Miller 2001). Viability can relatively easily be assessed by the culture method or by flow cytometry (Virta et al. 1998; Bunthof et al. 2001). It is important to guarantee the viability of probiotics in the final product especially when viability has been documented as one of prerequisites for immune effects (Gill & Rutherfurd 2001).

Future probiotics

Probiotics for specific target groups

Current probiotics have mainly been selected based on the common criteria as outlined in Table 2. To refine the selection criteria, understanding of the mechanisms of probiotic action is necessary. This will make it possible to select future strains with more specific characteristics, to suit the needs of specific age and patient groups. This need is clearly indicated by the difference in mucosal adhesion of probiotic bifidobacteria to mucus from different age groups (Ouwehand et al. 1999) and the influence of disease on mucosal adhesion of selected probiotics (Ouwehand et al. 2002). The use of specially selected probiotics for particular subject groups may provide more specific health effects.

Non-viable probiotics

Most definitions of probiotic bacteria stress the importance of the viability of the microbes. However, very little research has been done on non-viable probiotics. Non-viable probiotics would have several advantages over viable ones: longer shelf life, improved safety and no need for refrigerated storage or transport. Review of the literature suggests that non-viable probiotics may have positive health effects as well (Ouwehand & Salminen 1998). This has been shown for shortening of rotavirus diarrhoea (Kaila et al. 1995) and alleviation of lactose intolerance (Vesa et al. 2000). Although viable probiotics appear to have more health effects than non-viable ones, the latter are not always without health effects. This also implicates that heat-inactivated products can not be used as controls without verifying their lack of activity.

Alternative applications

Probiotics are mainly used to influence the composition or activity of the intestinal microflora. However, in principle any part of the body which harbours a normal microflora can be a potential target for specific probiotics.

The oral cavity has a microflora that equals the intestinal microflora in complexity. Here too, some of the members of the normal microflora have a detrimental effect on the host, causing, e.g. dental caries or periodontal disease. Probiotics could have potential applications in the oral cavity. Yoghurt have been observed to reduce the colonisation by mutans streptococci, which are responsible for dental caries (Petti et al. 2001). While a specific probiotic *Lactobacillus* strain has been detected in saliva samples (Meurman et al. 1994). Although there is a considerable potential for probiotic use in the oral cavity, very little work has been done in this area.

The normal microflora of the urogenital tract is less complex than the microflora of the intestine and the oral cavity. However, more than 50 species are thought to colonise the urogenital tract and in health, H_2O_2-producing lactobacilli predominate (Redondo-Lopez et al. 1990). Disturbances in the *Lactobacillus*

flora are thought to be related to the risk for urinary tract infections. Some work has therefore been done on the use of probiotics for urogenital tract infections. Selected *Lactobacillus* strains have been observed to reduce the recurrence of urinary tract infections (Reid et al. 1992) and reduce the risk for vaginitis (Hilton et al. 1992; Reid et al. 2001). Much work has also been done on the mechanisms of probiotic lactobacilli on urinary tract infections; production of hydrogen peroxide and of biosurfactants appear to be important factors contributing to the efficacy of the probiotic strains for use in the urogenital tract (Reid 2001). The probiotic *L. casei* Shirota has been observed to reduce the recurrence of superficial bladder cancer (Aso et al. 1995). These findings indicate that use of probiotics for the urogenital tract is a promising future area.

The skin has a normal microflora which is different depending on the site of the body. The most common genera found in the microflora of the skin are propionibacteria, *Staphylococcus, Micrococcus, Corynebacterium* and the yeast *Malassezia*. Several species within these genera can be opportunistic pathogens. However, the potential use of probiotics for the skin has been considered little to non (Barefoot & Ratnam 1998).

Also, the nasopharynx has a normal microflora, *Streptococcus pneumoniae* being frequently one of its normal members. Even lactobacilli have been isolated from the upper respiratory tract. Their potential use as probiotics in there has only recently been considered and may have interesting applications (Cangemi de Gutierrez et al. 2001).

Thus, there are many potential applications for probiotics which have received little attention but which may provide significant health effects.

Conclusion

The specific health effects of selected probiotic strains are becoming increasingly accepted thanks to an expanding volume of documentation from double-blind, placebo-controlled, clinical studies. In particular, relief of lactose intolerance symptoms, by yoghurt cultures, shortening of rotavirus diarrhoea and treatment of allergies are now well established. Also, the mechanisms behind these health effects are being elucidated through *in vitro* and animal studies, this can be expected to lead to more carefully formulated selection criteria for probiotics. However, many proposed beneficial health effects of probiotics still need further investigation, in particular the potential benefits for healthy consumers. For this, it is important to use well defined strains, since each strain has to be judged on its own merits, and that appropriate biomarkers are used for the evaluation of the effects. In addition to this, well selected target groups are needed. Such studies may indicate additional areas for probiotic use and further consolidate the acceptance of probiotics.

References

Aso Y, Akaza H, Kotake T, Tsukamoto T, Imai K, Naito S & BLP Study Group (1995) Preventive effect of a *Lactobacillus casei* preparation on the recurrence of superficial bladder cancer in a double-blind trial. Eur. J. Urol. 27: 104–109.

Barefoot SF & Ratnam P (1998) Composition for treating acne. US patent WO98/10743.

Benno Y, Mitsuoka T & Kanazawa K (1991) Human faecal flora in health and colon cancer. Acta Chirurgica Scand. 521: 15–23.

Benno Y, He F, Hosoda M, Hashimoto H, Kojima T, Yamazaki K, Iino H, Mykkänen H & Salminen S (1996) Effects of *Lactobacillus* GG yogurt on human intestinal microecology in Japanese subjects. Nutrition Today 31: 9S–11S.

Black FT, Andersen PL, Örsskov J, Örskov F, Gaarslev K & Laulund S (1989) Prophylactic efficacy of lactobacilli on travelers diarrhea. Travel Med. 7: 333–335.

Black F, Einarsson K, Lidbeck A, Orrhage K & Nord CE (1991) Effect of lactic acid producing bacteria on the human intestinal microflora during ampicillin treatment. Scand. J. Infect. Dis. 2: 247–254.

Brandtzaeg P (1995) Molecular and cellular aspects of the secretory immunoglobulin system. APMIS 103: 1–19.

Brigidi P, Vitali B, Swennen E, Bazzocchi G & Matteuzzi D (2001) Effects of probiotic administration upon the composition and enzymatic activity of human fecal microbiotia in patients with irritable bowel syndrome or functional diarrhea. Res. Microbiol. 152: 735–741.

Bunthof CJ, Bloemen K, Breeuwer P, Rombouts FM & Abee T (2001) Flow cytometric assessment of viability of lactic acid bacteria. Appl. Environ. Microbiol. 67: 2326–2335.

Cangemi de Gutierrez R, Santos V & Nader-Macías, ME (2001) Protective effect of intranasally inocculated *Lactobacillus fermentum* against *Streptococcus pneumoniae* challenge on the mouse respiratory tract. FEMS Immunol. Med. Microbiol. 31: 187–195.

Claeson M & Merson MH (1990) Global progress in the control of diarrheal disease. Pediatr. Infect. Dis. J. 9: 345–355.

Ducroc R, Heyman M, Beaufrere B, Morgat JL & Desjeux JF (1983) Horseradish peroxidase transport across rabbit jejunum and Peyer's patches in vitro. Am. J. Physiol. 245: G54–G58.

Elo S, Saxelin M & Salminen S (1991) Attachment of *Lactobacillus casei* strain GG to human colon carcinoma cell line Caco-2: comparison with other dairy strains. Lett. Appl. Microbiol. 13: 154–156.

Felley CP, Corthesy-Theulaz I, Rivero JL, Sipponen P, Kaufmann M, Bauerfeind P, Wiesel PH, Brassart D, Pfeifer A, Blum AL & Michetti P (2001) Favourable effect of an acidified milk (LC-1) on *Helicobacter pylori* gastritis in man. Eur. J. Gastroenterol. Hepatol. 13: 25–29.

Fuller R (1989) Probiotics in man and animals. J. Appl. Bacteriol. 66: 365–378.

Gill HS & Rutherfurd KJ (2001) Probiotic supplementation to enhance natural immunity in the elderly: effects of a newly characterized immunomodulatory strain *Lactobacillus rhamnosus* HN001 (DR20^{TM}) on leucocyte phagocytosis. Nutr. Res. 21: 183–189.

Gismondo MR, Drago L & Lombardi A (1999) Review of probiotics available to modify gastrointestinal flora. Int. J. Antimicrobial Agents 12: 287–292.

Goldin BR (1998) Health benefits of probiotics. Br. J. Nutr. 80: S203–S207.

Greenwald P, Clifford CK & Milner JA (2001) Diet and cancer prevention. Eur. J. Cancer 37: 948–965.

Grönlund MM, Arvilommi H, Kero P, Lehtonen OP & Isolauri E (2000) Importance of intestinal colonisation in the maturation of humoral immunity in early infancy: a prospective follow up study of healthy infants aged 0–6 months. Arch. Dis. Childhood 83: F186–F192.

Groux H & Powrie F (1999) Regulatory T cells and inflammatory bowel disease. Immunol. Today 20: 442–446.

Guandalini S, Pensabene L, Zikri MA, Dias JA, Casali LG, Hoekstra H, Kolacek S, Massar K, Micetic-Turk D, Papadopoulou A, de Sousa JS, Sandhu B, Szajewska H & Weizman Z (2000) *Lactobacillus* GG administered in oral rehydration solution to children with acute diarrhea: a multicenter European trial. J. Ped. Gastroenterol. Nutr. 30: 54–60.

Guarner F & Schaafsma GJ (1998) Probiotics. Int. J. Food Microbiol. 39: 237–238.

Gupta P, Andrew H, Kirschner BS & Guandalini S (2000) Is *Lactobacillus* GG helpful in children with Crohn's disease? Results of a preliminary, open-label study. J. Ped. Gastroenterol. Nutr. 31: 453–457.

Guslandi M, Mezzi G, Sorghi M & Testoni PA (2000) *Saccharomyces boulardii* in maintenance treatment of Crohn's disease. Digestive Dis. Sci. 45: 1462–1464.

Haller D, Bode C, Hammes WP, Pfeifer AMA, Schiffrin EJ & Blum S (2000) Non-pathogenic bacteria elicit differential cytokine response by intestinal epithelial cell/Leukocyte co-cultures. Gut 47: 79–87.

Hamilton-Miller JMT (2001) A review of clinical trials of probiotics in the management of inflammatory bowel diesease. Infect. Dis. Rev. 3: 83–87.

Hamilton-Miller JMT & Shah S (2002) Deficiencies in microbiological quality and labellinf of probiotic supplements. Int. J. Food Microbiol. 72: 175–176.

Hatakka K, Savilahti E, Pönkä A, Meurman JH, Poussa T, Näse L, Saxelin M & Korpela R (2001) Effect of long term consumption of probiotic milk on infections in children attending day care centres: double blind randomised trial. Br. Med. J. 322: 1327–1329.

Helgeland L, Vaage JT, Rolstad B, Midtvedt T & Brandtzaeg P (1996) Microbial colonization influences composition and T-cell receptor V beta repertoire of intraepithelial lymphocytes in rat intestine. Immunology 89: 494–501.

Heyman M & Desjeux JF (2000) Cytokine-induced alteration of the epithelial barrier to food antigens in disease. Ann. NY Acad. Sci. 915: 304–311.

Hilton E, Isenberg HD, Alperstein P, France K & Borenstein MT (1992) Ingestion of yoghurt containing *Lactobacillus acidophilus* as prophylaxis for candidal vaginitis. Ann. Int. Med. 116: 353–357.

Hirayama K & Rafter J (2000) The role of probiotic bacteria in cancer prevention. Microbes Infect. 2: 681–686.

Isolauri E (1999) Probiotics and gut inflammation. Curr. Opin. Gastroenterol. 15: 534–537.

Isolauri E (2001) Probiotics in human disease. Am. J. Clin. Nutr. 73: S1142–S1146.

Isolauri E, Kaila M, Arvola T, Majamaa H, Rantala I, Virtanen E & Arvilommi H (1993) Diet during rotavirus enteritis affects jejunal permeability to macromolecules in suckling rats. Ped. Res. 33: 548–553.

Isolauri E, Arvola T, Sütas Y , Moilanen E & Salminen S (2000) Probiotics in the management of atopic eczema. Clin. Exp. Allergy 30: 1605–1610.

Isolauri E, Sütas Y, Kankaanpää P, Arvilommi H & Salminen S (2001) Probiotics: effects on immunity. Am. J. Clin. Nutr. 73: S444–S445.

Kaila M, Isolauri E, Soppi E, Virtanen E, Laine S & Arvilommi H (1992) Enhancement of the circulating antibody secreting cell response in human diarrhea by a human *Lactobacillus* strain. Ped. Res. 32: 141–144.

Kaila M, Isolauri E, Saxelin M, Arvilommi H & Veskari T (1995) Viable versus inactivated *Lactobacillus* strain GG in acute rotavirus diarrhoea. Arch. Dis. Childhood 72: 51–53.

Kalliomäki M, Kirjavainen P, Eerola E, Kero P, Salminen S & Isolauri E (2001a) Distinct patterns of neonatal gut microflora in infants in whom atopy was and was not developing. J. Allergy Clin. Immunol. 107: 129–134.

Kalliomäki M, Salminen S, Arvilommi H, Kero P, Koskinen P & Isolauri E (2001b) Probiotics in primary prevention of atopic disease: a randomised placebo-controlled trial. Lancet 357: 1076–1079.

Lasser RB, Bond JH & Levitt MD (1975) The role of intestinal gas in functional abdominal pain. New Engl. J. Med. 293: 524–526.

Launiala K (1968) The effect of unabsorbed sucrose and mannitol on the small intestinal flow rate and mean transit time. Scand. J. Gastroenterol. 3: 665–671.

Lee Y-K, Nomoto K, Salminen S & Gorbach SL (1999) Handbook of Probiotics. John Wiley & Sons, Inc., New York.

Link-Amster H, Rochat F, Saudan KY, Mignot O, Aeschlimann JM (1994) Modulation of a specific humoral immune response and changes in intestinal flora mediated through fermented mik intake. FEMS Immunol. Med. Microbiol. 10: 55–64.

MacDonald T (2001) The reaction of the immune system to pathogens but not food antigens and commensal bacteria. Seminars Immunol. 13: 159–161.

Majamaa H & Isolauri E (1997) Probiotics: a novel approach in the management of food allergy. J. Allergy Clin. Immunol. 99: 179–186.

Malchow HA (1997) Crohn's disease and *Echerichia coli*. J. Clin. Gastroenterol. 25: 653–658.

Mattila-Sandholm T, Blum S, Collins JK, Crittenden R, de Vos W, Dunne C, Fondén R, Grenov G, Isolauri E, Kiely B, Marteau P, Morelli L, Ouwehand A, Reniero R, Saarela M, Salminen S, Saxelin M, Schiffrin E, Shanahan F, Vaughan E & von Wright A (1999) Probiotics: towards demonstrating efficacy. Trends Food Sci. Technol. 10: 393–399.

Meurman JH, Antila H & Salminen S (1994) Recovery of *Lactobacillus* strain GG (ATCC 53103) from saliva of healthy volunteers after consumption of yoghurt prepared with the bacterium. Microbial Ecol. Health Dis. 7: 295–298.

Nagao F, Nakayama M, Muto T & Okumura K (2000) Effects of a fermented milk drink containing *Lactobacillus casei* strain Shirota on the immune system in healthy human subjects. Biosci. Biotechnol. Biochem. 64: 2706–2708.

Niedzielin K, Kordecki H & Birkenfeld B (2001) A controlled, double-blind, randomized study on the efficacy of *Lactobacillus*

plantarum 299v in patients with irritable bowel syndrome. Eur. J. Gastroenterol. Hepatol. 13: 1143–1147.

Ouwehand AC & Salminen SJ (1998) The health effects of cultured milk products with viable and non-viabel bacteria. Int. Dairy J. 8: 749–758.

Ouwehand AC, Isolauri E, Kirjavainen PV & Salminen SJ (1999) Adhesion of four *Bifidobacterium* strains to human intestinal mucus from subjects in different age groups. FEMS Microbiol. Lett. 172: 61–64.

Ouwehand AC, Tölkkö S & Salminen S (2001) The effect of digestive enzymes on the adhesion of probiotic bacteria *in vitro*. J. Food Sci. 66: 856–859.

Ouwehand AC, Salminen S, Tölkkö S, Roberts PJ, Ovaska J & Salminen E (2002) Disease dependent adhesion of lactic acid bacteria to colonic tissue *in vitro*. Microecol. Ther. In press.

Pessi T, Isolauri E, Sütas Y, Kankaanranta H, Moilanen E & Hurme M (2000a) Suppression of T cell activation by *Lactobacillus rhamnosus* GG-degraded bovine casein. Immunopharmacology 1: 211–218.

Pessi T, Sütas Y, Hurme M & Isolauri E (2000b) Interleukin-10 generation in atopic children following oral *Lactobacillus rhamnosus* GG. Clin. Exp. Allergy 30: 1804–1808.

Petti S, Tarsitani G & Simonetti D'Arca A (2001) A randomized clinical trial of the effect of yoghurt on the human salivary microflora. Arch. Oral Biol. 46: 705–712.

Redondo-Lopez V, Cook RL & Sobel JD (1990) Emerging role of lactobacilli in the control and maintenance of the vaginal bacterial microflora. Rev. Infect. Dis. 12: 856–872.

Reid G, Bruce AW & Taylor M (1992) Influence of three day antimicrobial therapy and *Lactobacillus* vaginal suppositories on recurrence of urinary tract infections. Clin. Therapy 14: 11–16.

Reid G (2001) Probiotic agents to protect the urogenital tract against infection. Am. J. Clin. Nutr. 73: 437S–443S.

Reid G, Bruce AW, Fraser N, Heinemann C, Owen J & Henning B (2001) Oral probiotics can resolve urogenital infections. FEMS Immunol. Med. Microbiol. 30: 49–52.

Saavedra JM, Bauman NA, Oung I, Perman JA & Yolken RH (1994) Feeding of *Bifidobacterium bifidum* and *Streptococcus thermophilus* to infants in hospital for prevention of diarrhoea and shedding of rotavirus. Lancet 344: 1046–1049.

Salim AF, Phillips AD & Farthing MJ (1990) Pathogenesis of gut virus infection. Baillières Clin. Gastroenterol. 4: 593–607.

Salminen S, Isolauri E & Salminen E (1996) Clinical uses of probiotics for stabilizing the gut mucosal barrier: successful strains and future challenges. Antonie Van Leeuwenhoek 70: 347–358.

Salminen S, Bouley C, Boutron-Ruault M-C, Cummings JH, Franck A, Gibson GR, Isolauri E, Moreau M-C, Roberfroid M & Rowland I (1998) Functional food science and gastrointestinal physiology and function. Br. J. Nutr. 80: S147–S171.

Salminen S, Ouwehand A, Benno Y & Lee YK (1999) Probiotics: how should they be defined? Trends Food Sci. Technol. 10: 107–110.

Sanderson IR & Walker WA (1993) Uptake and transport of macromolecules by the intestine: possible role in clinical disorders (an update). Gastroenterology 104: 622–639.

Shimoyama T, Hori S, Tamura K, Yamamura M, Tanaka M & Yamazaki K (1984) Microflora of patients with stool abnormality. Bifidobacteria and Microflora 3: 35–42.

Shornikova A-V, Casas I, Mykkänen H, Salo E & Vesikari T (1997) Bacteriotherapy with *Lactobacillus reuteri* in rotavirus gastroenteritis. Ped. Infect. Dis. J. 16: 1103–1107.

Spanhaak S, Havenaar R & Schaafsma G (1998) The effect of consumption of milk fermented by *Lactobacillus casei* strain Shirota on the intestinal microflora and immune parameters in humans. Eur. J. Clin. Nutr. 52: 899–907.

Strachan DP (1989) Hay fever, hygiene, and household size. Br. Med. J. 299: 1259–1260.

Sudo N, Sawamura S, Tanaka K, Aiba Y, Kubo C & Koga Y (1997) The requirement of intestinal bacterial flora for the development of an IgE production system fully susceptible to oral tolerance induction. J. Immunol. 159: 1739–1745.

Sugita T & Togawa M (1994) Efficacy of *Lactobacillus* preparation Biolactis powder in children with rotavirus enteritis. Jpn. J. Pediatr. 47: 2755–2762.

Surawicz CM, Elmer GW, Spleeman P, McFarland LV, Chinn J & van Belle G (1989) Prevention of antibiotic associated diarrhoea by *Saccharomyces boulardii*: a prospective study. Gastroenterology 96: 981–988.

Sütas Y, Hurme M & Isolauri E (1996) Downregulation of antiCD3 antibody-induced IL-4 production by bovine caseins hydrolysed with *Lactobacillus* GG-derived enzymes. Scand. J. Immunol. 43: 687–689.

Temmerman R, Huys G, Pot B & Swings J (2001) Identification and antibiotic resistance of isolates from probiotic products. Abstracts of 101st ASM General Meeting, C-289.

Tuomola EM, Ouwehand AC & Salminen SJ (2000) Chemical, physical and enzymatic pre-treatments of probiotic lactobacilli alter their adhesion to human intestinal mucus glycoproteins. Int. J. Food Microbiol. 60: 75–81.

Tuomola E, Crittenden R, Playne M, Isolauri E & Salminen S (2001) Quality assurence criteria for probiotic bacteria. Am. J. Clin. Nutr. 73: S393–S398.

Vesa T, Marteau P & Korpela R (2000) Lactose intolerance. J. Am. Coll. Nutr. 19: 165S–175S.

Virta M, Lineri S, Kankaanpää P, Karp M, Peltonen K, Nuutila J & Lilius E-M (1998) Determination of complement-mediated killing of bacteria by viability staining and bioluminescence. Appl. Environ. Microbiol. 64: 515–519.

frontiers in Microbiology

MINI REVIEW
published: 08 January 2016
doi: 10.3389/fmicb.2015.01522

Opportunistic Strains of *Saccharomyces cerevisiae*: A Potential Risk Sold in Food Products

Roberto Pérez-Torrado† *and Amparo Querol**†

Food Biotechnology Department, Instituto de Agroquímica y Tecnología de los Alimentos – Consejo Superior de Investigaciones Científicas, Valencia, Spain

In recent decades, fungal infections have emerged as an important health problem associated with more people who present deficiencies in the immune system, such as HIV or transplanted patients. *Saccharomyces cerevisiae* is one of the emerging fungal pathogens with a unique characteristic: its presence in many food products. *S. cerevisiae* has an impeccably good food safety record compared to other microorganisms like virus, bacteria and some filamentous fungi. However, humans unknowingly and inadvertently ingest large viable populations of *S. cerevisiae* (home-brewed beer or dietary supplements that contain yeast). In the last few years, researchers have studied the nature of *S. cerevisiae* strains and the molecular mechanisms related to infections. Here we review the last advance made in this emerging pathogen and we discuss the implication of using this species in food products.

Keywords: yeast, *S. cerevisiae*, food, opportunistic, infection

OPEN ACCESS

Edited by:
Amit Kumar Tyagi,
The University of Texas MD Anderson Cancer Center, USA

Reviewed by:
Catarina Prista,
Instituto Superior de Agronomia, Portugal
Matthias Sipiczki,
University of Debrecen, Hungary

****Correspondence:***
Amparo Querol
aquerol@iata.csic.es

†These authors have contributed equally to this work

Specialty section:
This article was submitted to Food Microbiology, a section of the journal Frontiers in Microbiology

Received: *15 October 2015*
Accepted: *17 December 2015*
Published: *08 January 2016*

Citation:
Pérez-Torrado R and Querol A (2016) Opportunistic Strains of Saccharomyces cerevisiae: A Potential Risk Sold in Food Products. Front. Microbiol. 6:1522. doi: 10.3389/fmicb.2015.01522

INTRODUCTION

Fungal infections are an extremely important health problem. According to numerous studies, *Candida albicans* and other *Candida* species are the most remarkable pathogenic fungi which cause some 7000-28000 nosocomial infections annually (Pfaller and Diekema, 2007). The general characteristic of fungal infection is that it is produced as a result of reduced immunity. Most fungal pathogens are classified as opportunistic. This concept implies that under normal conditions, these organisms are not capable of producing infection but, when host defenses are weakened, there is room for them to prosper and to generate a health problem. Another general characteristic of fungal infections is that they are frequently moderated and localized. However, fungal pathogens are able to produce fungal disease, systemic infection, and even death in the worst scenarios.

In the last century, fungal infection cases have dramatically increased, especially in developed countries. One work has shown that the number of cases of sepsis produced by fungal organisms in the USA has increased by 207% between 1979 and 2000 (Martin et al., 2003). This phenomenon is associated with the appearance of medical techniques, such as the use of broad-spectrum antibiotics, the use of intravenous catheters, how intensive care units are organized, increased number of organ transplants, or the development of cytotoxic chemotherapies. On top of that, pandemics like HIV/AIDS have exponentially increased the number of patients with impaired immunity. In fact, fungal disease was extremely rare before all these changes occurred.

A paradigm of an emerging fungal organism is the yeast *Saccharomyces cerevisiae*. This species can be found naturally in many niches in the environment, but is most commonly known for its

role as "baker's yeast" in either traditional or industrial fermentative production of bread, beer or wine. It has also been used as an agent to treat antibiotic-related diarrhea and as a nutritional supplement, when it is commercialized as *S. boulardii*. Classically, *S. cerevisiae* has been considered a safe non pathogenic organism. However in the last two decades, the number of cases of diagnosed infections has increased, probably as a result of the increased numbers of immunocompromised patients, but also due to advances made in diagnostic methodologies in hospitals, including genetic identification by molecular techniques. *S. cerevisiae* has been related to a wide variety of infections, which range from vaginitis in healthy patients and cutaneous infections, to systemic bloodstream infections and infections of essential organs in immunocompromised and critically ill patients (Enache-Angoulvant and Hennequin, 2005; Muñoz et al., 2005; de Llanos et al., 2011). Infected patients tend to be elderly people, premature children or patients suffering from immunosuppression due to HIV/AIDS, treatment with immunosuppressive agents, or other conditions associated with a deficient immune response. Furthermore, severe infections with *S. cerevisiae* have been occasionally reported in patients with no obvious predisposing factors (Jensen and Smith, 1976; Smith et al., 2002). All these data have changed the status of *S. cerevisiae*, which is now considered an emerging opportunistic pathogen (Herbrecht and Nivoix, 2005; de Llanos et al., 2006).

S. cerevisiae POPULATION DIVERSITY: OPPORTUNISTIC STRAINS

The species *S. cerevisiae* is very heterogeneous and contains strains with specific abilities like sherry wine strains, *S. boulardii* or baker strains. Before the development of high throughput sequencing techniques, the population structure of *S. cerevisiae* was not very clear. Now we know that it is structured into several genetically pure subpopulations and many mosaic strains that contain gene alleles of different subpopulations (Liti et al., 2009). In the last decade, yeast scientists have attempted to determine if the strains isolated from infected patients form a specific *S. cerevisiae* subpopulation with any special characteristic. de Llanos et al. (2004) used molecular markers as mt DNA restriction patterns and showed that clinical strains were present in several genetically differentiated groups of strains. In contrast, Carreto et al. (2008) used comparative genome hybridization on array (aCGH), and suggested that clinical strains could be a genetically homogenous subpopulation. Later, Wei et al. (2007) sequenced the genome of *S. cerevisiae* strain YJM789 derived from a yeast isolated from the lung of an AIDS patient with pneumonia. Liti et al. (2009) sequenced 36 new strains that contained six clinical isolates. Strope et al. (2015) sequenced 93 strains from multiple geographic and environmental origins, including several clinical strains. Finally, after the sequencing of the whole genome of several clinical strains, it turns out that they are not a genetically homogenous group of strains, but are all mosaic strains with relatively heterogeneous genetic content (Liti et al., 2009; Strope et al., 2015).

Several studies have analyzed the potential virulence of this yeast species *in vitro* (McCusker et al., 1994; de Llanos et al., 2006) and *in vivo* (McCullough et al., 1998; de Llanos et al., 2011), and have suggested that some strains have the potential to cause disease regardless of their clinical or non clinical isolation origin. Many strains of *S. cerevisiae,* which have been isolated in clinical settings, present very low levels of virulence in mice infection models, while other strains, such as strain D14 isolated from a dietetic supplement, have shown a relative high level of virulence in different infection models (Llopis et al., 2012, 2014; Pérez-Torrado et al., 2015). Thus we propose that the term "opportunistic strain" is used more accurately and usefully to categorize these strains rather than "clinical strains" since not all clinical strains cause infections. Even more importantly, some non clinical strains can cause infections. We define opportunistic *S. cerevisiae* strains as those strains which show physiologic characteristics of yeast pathogens, such as growth, at 37 °C (McCusker et al., 1994; de Llanos et al., 2006), but unlike most other strains, can also cause infections and kill mice (de Llanos et al., 2011). These strains also survive better in human blood infection models than other strains, which may enable them to disseminate across the body and reach organs under adequate propagation conditions and in certain circumstances (Lin et al., 2012). Increased blood survival can be a key feature to distinguish these opportunistic yeasts strains from others.

S. cerevisiae INFECTION MECHANISMS

The infection mechanism of *C. albicans*, a fungal pathogen that is phylogenetically close to *S. cerevisiae*, is based on a first step of adhesion to human surface tissues and uses a family of proteins called adhesins. *C. albicans* is also able to penetrate epithelial or endothelial barriers via active mechanisms (Dalle et al., 2010). A second important aspect of infection mechanisms is resistance to the stressful situations generated after an encounter with cells of the immune system. In contrast, *S. cerevisiae* lacks homologous genes for the well-known *C. glabrata* or *C. albicans* adhesins and shows low adhesion levels to human tissues compared to both *Candida* species (Pérez-Torrado et al., 2012). It has been suggested that *S. cerevisiae* can only perform opportunistic or passive crossings when epithelial barrier integrity is previously compromised (Pérez-Torrado et al., 2012).

However, previous studies by our group (Llopis et al., 2012) have demonstrated that opportunistic *S. cerevisiae* strains show a specific transcription pattern after human blood infection, which reflects a specific oxidative stress response, increased amino acid biosynthesis and a DNA damage repair response (**Figure 1**).

Yeast pathogens are adapted to resist human defenses and one of the main responses of the immune system is microbe engulfment and oxidative burst. Neutrophils, macrophages and other cells with phagocytic capacity generate potent reactive oxygen and nitrogen species (ROS and RNS), which can be lethal to most fungal pathogens by causing damage to DNA, proteins and lipids (Bogdan et al., 2000). Most fungal pathogens display resistance to the reactive oxygen and nitrogen species

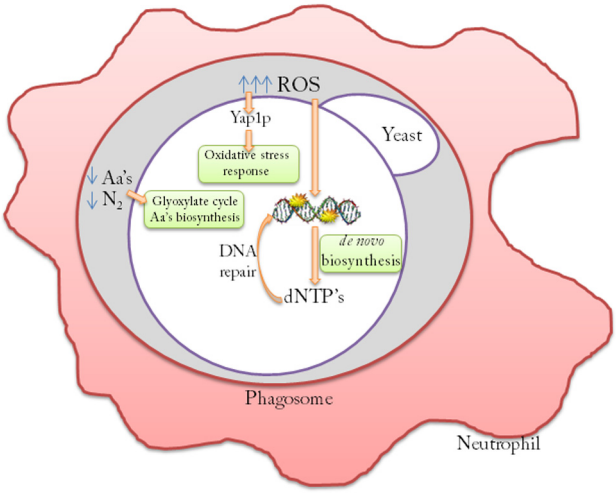


FIGURE 1 | Molecular mechanisms of persistence of *Saccharomyces cerevisiae* cells after neutrophil engulfment. Opportunistic *S. cerevisiae* strains redirect the low levels of the nitrogen sources inside the phagosome to increase amino acids (aa's) biosynthesis. These strains also increase the oxidative stress response by activating the Yap1p transcription factor regulon to counteract the lethal effects of reactive oxygen species (ROS) generated by the macrophage. *De novo* dNTP biosynthesis is activated to allow DNA repair machinery to counteract ROS-induced mutations.

used by human cells to counteract infection (Brown et al., 2009). Fungal resistance to ROS offers protection from oxidative host defenses and is undoubtedly an advantageous pathobiological property (Lushchak et al., 2010; Rodrigues-Pousada et al., 2010). It has been described that pathogens such as *C. albicans* or *C. neoformans* have the potential to resist, among other suboptimal conditions, oxidative stress produced by the ROS generated in the phagosome (Brown et al., 2007, 2014). Indeed the thioredoxin system of *C. albicans* and *S. cerevisiae* has been shown to be expressed during growth in human blood or mucosal tissue (Fradin et al., 2003, 2005, Zakikhany et al., 2007; Llopis et al., 2012), which indicates that the ability to respond to oxidative stress might be crucial in early stages of systemic infections. *TRX1* (thioredoxin 1) is also necessary to survive the oxidative environment of macrophages in *C. neoformans*, and is important for the virulence of this fungal pathogen (Missall and Lodge, 2005). The *TSA2* (thioredoxin peroxidase 2) and *GPX2* (glutathione peroxidase 2) genes have been shown to be induced in *S. cerevisiae* strains when exposed to neutrophils (Rubin-Bejerano et al., 2003), and a clear antioxidant response has been observed. Fradin et al. (2005) demonstrated that neutrophils play a key role in bloodstream infections with *C. albicans*. This observation is in line with the high susceptibility shown by neutropenic patients (deficient in these immune cells) to disseminated candidiasis (Bodey et al., 1992; Wright and Wenzel, 1997). In *S. cerevisiae,* the main role of proper oxidative stress response virulence has been suggested since a virulent strain mutant in transcription factor Yap1p, the main transcription factor involved in oxidative stress response that is unable to grow under oxidative stress conditions, presented low survival levels in human blood compared with the wild type or the *YAP1* reconstituted strain (Llopis et al., 2012). Diezmann and Dietrich (2011) compared hundreds of clinical isolates and showed that they were more resistant to oxidative stress after they verified the central role of oxidative stress resistance in *S. cerevisiae* virulence.

After exposure to human neutrophils or cultured macrophages, *C. albicans* cells up-regulate amino acid biosynthetic genes (Rubin-Bejerano et al., 2003; Fradin et al., 2005). Rubin-Bejerano et al. (2003) observed induction for these pathways after *C. albicans* and *S. cerevisiae* cells were ingested by neutrophils. This suggests that the microenvironment in the phagosome inside the neutrophil is deficient in amino acids, and generates a rapid response from yeast strains. The methionine and arginine biosynthetic genes are induced when *S. cerevisiae* is phagocytized by the murine macrophage-like cell line (Lorenz and Fink, 2001). Kingsbury et al. (2006) revealed the relevance of amino acid biosynthesis for yeast survival in a murine host and suggested that yeast can use a variety of nitrogen sources under these conditions. The glyoxylate cycle is also induced upon phagocytes ingestion of the bacterium *Mycobacterium tuberculosis* (McKinney et al., 2000), and other fungi such as *C. neoformans* (Rude et al., 2002), *Leptosphaeria maculans* (Idnurm and Howlett, 2002), *C. albicans* (Lorenz and Fink, 2001), and *S. cerevisiae* (Lorenz and Fink, 2001). This change in metabolism is a response to the glucose-poor environment of the macrophage, and contributes to the virulence of some pathogens. The *ICL1* gene, which encodes for isocitrate lyase, one of the principal enzymes of the glyoxylate cycle, has been recently shown to be substantially induced upon exposure to macrophages *in vitro* in both *S. cerevisiae* and *C. albicans* (Lorenz and Fink, 2001; Lorenz et al., 2004). All these data suggest that the *ICL1* gene may also play a general role in *S. cerevisiae* in human infections.

A common mechanism described for human microbial pathogens is to increase dNTP pools in order to repair the DNA damage caused by the oxidative burst of phagocytes. The importance of a *de novo* nucleotide biosynthetic pathway for phagocyte survival has been demonstrated for bacterial pathogens such as *Salmonella enterica* (Panosa et al., 2010), *Bacteroides fragilis* (Smalley et al., 2002), *Pseudomonas aeruginosa* (Sjöberg and Torrents, 2011), *Staphylococcus aureus* (Kirdis et al., 2007), *Streptococcus pyogenes* (Le Breton et al., 2013), *Bacillus anthracis*, and *Escherichia coli* (Samant et al., 2008). The relevance of *de novo* nucleotide biosynthetic pathways has also been implicated for fungal pathogens like *Cryptococcus neoformans* (Morrow et al., 2012) or *C. albicans* (Donovan et al., 2001; Jiang et al., 2010), and nucleotide biosynthetic pathways have been discussed as a target for antifungal compounds (Rodriguez-Suarez et al., 2007). In a recent study, we observed that *S. cerevisiae* opportunistic strains, like other human pathogens, have the enhanced ability to produce dNTPs, the substrates used by DNA repair machineries (Pérez-Torrado et al., 2015). The importance of this pathway for the virulence of *S. cerevisiae* has been confirmed by experimental infections conducted in immunodeficient murine models using a Δ*gua1* mutant, which is a key enzyme for *de novo* dNTP biosynthesis. Additions of

exogenous guanine and the use of mutants in the DNA damage checkpoint, which activates dNTP biosynthesis in yeast cells, affects the survival of yeast cells in *ex vivo* blood infections (Pérez-Torrado et al., 2015). The nitrogen source preferentially used by yeasts in phagosomes to increase this pathway is still unknown.

CONCLUSION AND PERSPECTIVES

In developed countries, consumers have been driven to take a more critical attitude about what they eat and drink as a requirement of modern life. Food microbiologists are facing the huge challenge of regarding food freshness that is implicit in consumer demand for more natural products. Additive-free safer food with less severe processing that has a satisfactory shelf life and is easy to prepare is required given the greater awareness of nutrition and health. These changes in consumer preferences, which modify food processes, may have important consequences and could affect both food quality and safety. Yeasts, especially *S. cerevisiae*, have an impeccably good food safety record compared to other microorganisms like virus, bacteria and some filamentous fungi. However, humans unknowingly and inadvertently ingest large, viable populations of *S. cerevisiae* without them having adverse impacts on their health (e.g., yeasts in home-brewed beer, beers enriched by yeast or dietary supplements that contain yeast are very common today). Nevertheless, having an open mind about, and conducting vigilance of, yeasts and food-borne diseases are required. Compared with other microbial groups, yeasts are not seen as aggressive pathogens, but they are capable of causing human disease in opportunistic circumstances (Puig-Asensio et al., 2014). As we summarize in this paper, yeast researchers have made progress in understanding the nature and virulence mechanism in *S. cerevisiae* strains. In the future, more attention has to be paid to industrial practices that are more prone to generate opportunistic *S. cerevisiae* strains.

ACKNOWLEDGMENTS

RP-T was supported by CICYT grants (ref. AGL2012-39937-CO2-01 and -02) from the Spanish Ministry of Education and Science and FEDER. This work was supported by grant PROMETEO (project PROMETEO/2009/019) from the Generalitat Valenciana.

REFERENCES

Bodey, G., Bueltmann, B., Duguid, W., Gibbs, D., Hanak, H., Hotchi, M., et al. (1992). Fungal infections in cancer patients: an international autopsy survey. *Eur. J. Clin. Microbiol. Infect. Dis.* 11, 99–109. doi: 10.1007/BF01967060

Bogdan, C., Rollinghoff, M., and Diefenbach, A. (2000). Reactive oxygen and reactive nitrogen intermediates in innate and specific immunity. *Curr. Opin. Immunol.* 12, 64–76. doi: 10.1016/S0952-7915(99)00052-7

Brown, A. J., Budge, S., Kaloriti, D., Tillmann, A., Jacobsen, M. D., Yin, Z., et al. (2014). Stress adaptation in a pathogenic fungus. *J. Exp. Biol.* 217, 144–155. doi: 10.1242/jeb.088930

Brown, A. J., Haynes, K., and Quinn, J. (2009). Nitrosative and oxidative stress responses in fungal pathogenicity. *Curr. Opin. Microbiol.* 12, 384–391. doi: 10.1016/j.mib.2009.06.007

Brown, S. M., Campbell, L. T., and Lodge, J. K. (2007). *Cryptococcus neoformans*, a fungus under stress. *Curr. Opin. Microbiol.* 10, 320–325. doi: 10.1016/j.mib.2007.05.014

Carreto, L., Eiriz, M. F., Gomes, A. C., Pereira, P. M., Schuller, D., and Santos, M. A. (2008). Comparative genomics of wild type yeast strains unveils important genome diversity. *BMC Genomics* 9:524. doi: 10.1186/1471-2164-9-524

Dalle, F., Wachtler, B., L'Ollivier, C., Holland, G., Bannert, N., Wilson, D., et al. (2010). Cellular interactions of *Candida albicans* with human oral epithelial cells and enterocytes. *Cel. Microbiol.* 12, 248–271. doi: 10.1111/j.1462-5822.2009.01394.x

de Llanos, R., Fernandez-Espinar, M. T., and Querol, A. (2006). A comparison of clinical and food *Saccharomyces cerevisiae* isolates on the basis of potential virulence factors. *Antonie Van Leeuwenhoek* 90, 221–231. doi: 10.1007/s10482-006-9077-7

de Llanos, R., Llopis, S., Molero, G., Querol, A., Gi, C., and Fernandez-Espinar, M. T. (2011). In vivo virulence of commercial *Saccharomyces cerevisiae* strains with pathogenicity-associated phenotypical traits. *Int. J. Food. Microbiol.* 144, 393–399. doi: 10.1016/j.ijfoodmicro.2010.10.025

de Llanos, R., Querol, A., Planes, A. M., and Fernández-Espinar, M. T. (2004). Molecular characterization of clinical *Saccharomyces cerevisiae* isolates and their association with non-clinical strains. *Syst. Appl. Microbiol.* 27, 427–435. doi: 10.1078/0723202041438473

Diezmann, S., and Dietrich, F. S. (2011). Oxidative stress survival in a clinical *Saccharomyces cerevisiae* isolate is influenced by a major quantitative trait nucleotide. *Genetics* 188, 709–722. doi: 10.1534/genetics.111.128256

Donovan, M., Schumuke, J. J., Fonzi, W. A., Bonar, S. L., Gheesling-Mullis, K., Jacob, G. S., et al. (2001). Virulence of a phosphoribosyl aminoimidazole carboxylase-deficient *Candida albicans* strain in an immunosuppressed murine model of systemic candidiasis. *Infect. Immun.* 69, 2542–2548. doi: 10.1128/IAI.69.4.2542-2548.2001

Enache-Angoulvant, A., and Hennequin, C. (2005). Invasive *Saccharomyces* infection: a comprehensive review. *Clin. Infect. Dis.* 41, 1559–1568. doi: 10.1086/497832

Fradin, C., De Groot, P., MacCallum, D., Schaller, M., Klis, F., Odds, F. C., et al. (2005). Granulocytes govern the transcriptional response, morphology and proliferation of *Candida albicans* in human blood. *Mol. Microbiol.* 56, 397–415. doi: 10.1111/j.1365-2958.2005.04557.x

Fradin, C., Kretschmar, M., Nichterlein, T., Gaillardin, C., d'Enfert, C., and Hube, B. (2003). Stage-specific gene expression of *Candida albicans* in human blood. *Mol. Microbiol.* 47, 1523–1543. doi: 10.1046/j.1365-2958.2003.03396.x

Herbrecht, R., and Nivoix, Y. (2005). *Saccharomyces cerevisiae* fungemia: an adverse effect of *Saccharomyces boulardii* probiotic administration. *Clin. Infect. Dis.* 40, 1635–1637. doi: 10.1086/429926

Idnurm, A., and Howlett, B. J. (2002). Isocitrate lyase is essential for pathogenicity of the fungus *Leptosphaeria maculans* to canola (*Brassica napus*). *Eukaryot. Cell* 1, 719–724. doi: 10.1128/EC.1.5.719-724.2002

Jensen, D. P., and Smith, D. L. (1976). Fever of unknown origin secondary to brewer's yeast ingestion. *Arch. Intern. Med.* 136, 332–333. doi: 10.1001/archinte.1976.03630030064011

Jiang, L., Zhao, J., Guo, R., Li, J., Yu, L., and Xu, D. (2010). Functional characterization and virulence study of ADE8 and GUA1 genes involved in the de novo purine biosynthesis in *Candida albicans*. *FEMS Yeast Res.* 10, 199–208. doi: 10.1111/j.1567-1364.2009.00600.x

Kingsbury, J. M., Goldstein, A. L., and McCusker, J. H. (2006). Role of nitrogen and carbon transport, regulation, and metabolism genes for Saccharomyces cerevisiae survival in vivo. *Eukaryot. Cell* 5, 816–824. doi: 10.1128/EC.5.5.816-824.2006

Kirdis, E., Jonsson, I. M., Kubica, M., Potempa, J., Josefsson, E., and Masalha, M. (2007). *Ribonucleotide reductase* class III, an essential enzyme for the anaerobic

growth of *Staphylococcus aureus*, is a virulence determinant in septic arthritis. *Microb. Pathog.* 3, 179–188. doi: 10.1016/j.micpath.2007.05.008

Le Breton, Y., Mistry, P., Valdes, K. M., Quigley, J., Kumar, N., Tettelin, H., et al. (2013). Genome-wide identification of genes required for fitness of group a *Streptococcus* in human blood. *Infect. Immun.* 81, 862–875. doi: 10.1128/IAI.00837-12

Lin, L., Ibrahim, A. S., Baquir, B., Palosaari, A., and Spellberg, B. (2012). Luminescent-activated transfected killer cells to monitor leukocyte trafficking during systemic bacterial and fungal infection. *J. Infect. Dis.* 205, 337–347. doi: 10.1093/infdis/jir72522124127

Liti, G., Carter, D. M., Moses, A. M., Warringer, J., Parts, L., James, S. A., et al. (2009). Population genomics of domestic and wild yeasts. *Nature* 458, 337–341. doi: 10.1038/nature07743

Llopis, S., Hernández-Haro, C., Monteoliva, L., Querol, A., Molina, M., and Fernández-Espinar, M. T. (2014). Pathogenic potential of *Saccharomyces* strains isolated from dietary supplements. *PLoS ONE* 9:e98094. doi: 10.1371/journal.pone.0098094

Llopis, S., Querol, A., Heyken, A., Hube, B., Jespersen, L., Fernandez-Espinar, T., et al. (2012). Transcriptomics in human blood incubation reveals the importance of oxidative stress response in *Saccharomyces cerevisiae* clinical strains. *BMC Genomics* 13:419. doi: 10.1186/1471-2164-13-419

Lorenz, M. C., Bender, J. A., and Fink, G. R. (2004). Transcriptional response of *Candida albicans* upon internalization by macrophages. *Eukaryot. Cell* 3, 1076–1087. doi: 10.1128/EC.3.5.1076-1087.2004

Lorenz, M. C., and Fink, G. R. (2001). The glyoxylate cycle is required for fungal virulence. *Nature* 412, 83–86. doi: 10.1038/35083594

Lushchak, O. V., Inoue, Y., and Lushchak, V. I. (2010). Regulatory protein Yap1 is involved in response of yeast *Saccharomyces cerevisiae* to nitrosative stress. *Biochemistry (Mosc.)* 75, 629–664. doi: 10.1134/S00062979100 50135

Martin, G. S., Mannino, D. M., Eaton, S., and Moss, M. (2003). The epidemiology of sepsis in the United States from 1979 through 2000. *N. Engl. J. Med.* 348, 1546–1554. doi: 10.1056/NEJMoa022139

McCullough, M. J., Clemons, K. V., McCusker, J. H., and Stevens, D. A. (1998). Species identification and virulence attributes of *Saccharomyces boulardii* (nom. inval.). *J. Clin. Microbiol.* 36, 2613–2617.

McCusker, J. H., Clemons, K. V., Stevens, D. A., and Davis, R. W. (1994). *Saccharomyces cerevisiae* virulence phenotype as determined with CD-1 mice is associated with the ability to grow at 42 degrees C and form pseudohyphae. *Infect. Immun.* 62, 5447–5455.

McKinney, J. D., Honerzu, B. K., Munoz-Elias, E. J., Miczak, A., Chen, B., Chan, W. T., et al. (2000). Persistence of *Mycobacterium tuberculosis* in macrophages and mice requires the glyoxylate shunt enzyme isocitrate lyase. *Nature* 406, 735–738. doi: 10.1038/35021074

Missall, T. A., and Lodge, J. K. (2005). Function of the thioredoxin proteins in *Cryptococcus neoformans* during stress or virulence and regulation by putative transcriptional modulators. *Mol. Microbiol.* 57, 847–858. doi: 10.1111/j.1365-2958.2005.04735.x

Morrow, C. A., Valkov, E., Stamp, A., Chow, E. W., Lee, I. R., Wronski, A., et al. (2012). De novo GTP biosynthesis is critical for virulence of the fungal pathogen *Cryptococcus neoformans*. *PLoS Pathog.* 8:e1002957. doi: 10.1371/journal.ppat.1002957

Muñoz, P., Bouza, E., Cuenca-Estrella, M., Eiros, J. M., Perez, M. J., Sanchez-Somolinos, M., et al. (2005). *Saccharomyces cerevisiae* fungemia: an emerging infectious disease. *Clin. Infect. Dis.* 40, 1625–1634. doi: 10.1086/429916

Panosa, A., Roca, I., and Gibert, I. (2010). Ribonucleotide reductases of *Salmonella typhimurium* transcriptional regulation and differential role in pathogenesis. *PLoS ONE* 5:e11328. doi: 10.1371/journal.pone.0011328

Pérez-Torrado, R., Llopis, S., Jespersen, L., Fernández-Espinar, T., and Querol, A. (2012). Clinical *Saccharomyces cerevisiae* isolates cannot cross the epithelial barrier in vitro. *Int. J. Food Microbiol.* 157, 59–64. doi: 10.1016/j.ijfoodmicro.2012.04.012

Pérez-Torrado, R., Llopis, S., Perrone, B., Gómez-Pastor, R., Hube, B., and Querol, A. (2015). Comparative genomic analysis reveals a critical role of de novo nucleotide biosynthesis for *Saccharomyces cerevisiae* virulence. *PLoS ONE* 10:e0122382. doi: 10.1371/journal.pone.0122382

Pfaller, M. A., and Diekema, D. J. (2007). Epidemiology of invasive candidiasis: a persistent public health problem. *Clin. Microbiol. Rev.* 20, 133–163. doi: 10.1128/CMR.00029-06

Puig-Asensio, M., Padilla, B., Garnacho-Montero, J., Zaragoza, O., Aguado, J. M., Zaragoza, R., et al. (2014). Epidemiology and predictive factors for early and late mortality in *Candida bloodstream* infections: a population-based surveillance in Spain. *Clin. Microbiol. Infect.* 20, O245–O254. doi: 10.1111/1469-0691.12380

Rodrigues-Pousada, C., Menezes, R. A., and Pimentel, C. (2010). The Yap family and its role in stress response. *Yeast* 27, 245–258. doi: 10.1002/yea.1752

Rodriguez-Suarez, R., Xu, D., Veillette, K., Davison, J., Sillaots, S., Kauffman, S., et al. (2007). Mechanism-of-action determination of GMP synthase inhibitors and target validation in *Candida albicans* and *Aspergillus fumigatus*. *Chem. Biol.* 14, 1163–1175. doi: 10.1016/j.chembiol.2007.09.009

Rubin-Bejerano, I., Fraser, I., Grisafi, P., and Fink, G. R. (2003). Phagocytosis by neutrophils induces an amino acid deprivation response in *Saccharomyces cerevisiae* and *Candida albicans*. *Proc. Natl. Acad. Sci. U.S.A.* 100, 11007–11012. doi: 10.1073/pnas.1834481100

Rude, T. H., Toffaletti, D. L., Cox, G. M., and Perfect, J. R. (2002). Relationship of the glyoxylate pathway to the pathogenesis of *Cryptococcus neoformans*. *Infect. Immun.* 70, 5684–5694. doi: 10.1128/IAI.70.10.5684-5694.2002

Samant, S., Lee, H., Ghassemi, M., Chen, J., Cook, J. L., Mankin, A. S., et al. (2008). Nucleotide biosynthesis is critical for growth of bacteria in human blood. *PLoS Pathog.* 4:e37. doi: 10.1371/journal.ppat.0040037

Sjöberg, B. M., and Torrents, E. (2011). Shift in ribonucleotide reductase gene expression in *Pseudomonas aeruginosa* during infection. *Infect. Immun.* 79, 2663–2669. doi: 10.1128/IAI.01212-10

Smalley, D., Rocha, E. R., and Smith, C. J. (2002). Aerobic-type ribonucleotide reductase in the anaerobe *Bacteroides* fragilis. *J. Bacteriol.* 184, 895–903. doi: 10.1128/jb.184.4.895-903.2002

Smith, D., Metzgar, D., Wills, C., and Fierer, J. (2002). Fatal *Saccharomyces cerevisiae* aortic graft infection. *J. Clin. Microbiol.* 40, 2691–2692. doi: 10.1128/JCM.40.7.2691-2692.2002

Strope, P. K., Skelly, D. A., Kozmin, S. G., Mahadevan, G., Stone, E. A., Magwene, P. M., et al. (2015). The 100-genomes strains, an *S. cerevisiae* resource that illuminates its natural phenotypic and genotypic variation and emergence as an opportunistic pathogen. *Genome Res.* 25, 762–774. doi: 10.1101/gr.185538.114

Wei, W., McCusker, J. H., Hyman, R. W., Jones, T., Ning, Y., Cao, Z., et al. (2007). Genome sequencing and comparative analysis of *Saccharomyces cerevisiae* strain YJM789. *Proc. Natl. Acad. Sci. U.S.A.* 104, 12825–12830. doi: 10.1073/pnas.0701291104

Wright, W. L., and Wenzel, R. P. (1997). Nosocomial candida epidemiology, transmission, and prevention. *Infect. Dis. Clin. North. Am.* 11, 411–425. doi: 10.1016/S0891-5520(05)70363-9

Zakikhany, K., Naglik, J. R., Schmidt-Westhausen, A., Holland, G., Schaller, M., and Hube, B. (2007). In vivo transcript profiling of *Candida albicans* identifies a gene essential for interepithelial dissemination. *Cell. Microbiol.* 9, 2938–2954. doi: 10.1111/j.1462-5822.2007.01009.x

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Saudi J Kidney Dis Transpl 2014;25(6):1266-1269

Saudi Journal of Kidney Diseases and Transplantation

Case Report

Invasive *Saccharomyces cerevisiae* Infection: A Friend Turning Foe?

Unnikrishnan Pillai, Joe Devasahayam,Aparna Narayana Kurup, Alexandre Lacasse

Department of Internal Medicine, St. Mary's HealthCenter, St. Louis, MO, USA

ABSTRACT. We report a very rare case of acute pyelonephritis in a 51-year-old female with a history of chronic kidney disease (CKD) and diabetes caused by a normally benign and a well-known human commensal organism, *Saccharomyces cerevisiae* that is very often prescribed as a probiotic in modern medical practice. The causal role of *S. cerevisiae* was confirmed by its isolation in blood, urine, stool as well as vaginal swabs thus proving its virulent nature in suitable situations.

Introduction

S. cerevisiae, a non-spore forming yeast, also known as brewer's yeast or baker's yeast, is a common colonizer of the human respiratory, gastrointestinal and urinary tracts and is generally considered as a benign organism. Its role as a clinically important and invasive pathogen is not well known. However, cases have been reported to cause invasive diseases in the setting of chronic underlying diseases like malignancy, HIV/ AIDS or of bone marrow transplantation.[3,4] There are very few case reports on invasive *S. cerevisiae*, and it causing acute pyelonephritis probably has never been reported. Here we report a case of acute pyelonephritis in a 51 year old female with a history of chronic kidney disease (CKD) and diabetes.

Correspondence to:

Dr. Unnikrishnan Pillai,
Department of Internal Medicine,
St. Mary's HealthCenter, St. Louis,
MO, USA
E-mail: unnikrishnanpk@yahoo.com

Case Report

Our patient is a 51-year-old African-American female with history of stage 5 chronic kidney disease and diabetes mellitus who presented with a oneweek history of generalized weakness and worsening left flank pain, with fever, chills and burning pain during urination. Her medications included Amlodipine, Thiamine, Folic acid, Metoprolol, Sodium bicarbonate and insulin.

Physical examinations revealed a blood pressure of 140/90 mm Hg, pulse rate of 116/min, respiratory rate of 20/min and temperature of 110.1°F. The patient had hepatomegaly (6 cm) and left costo-vertebral angle tenderness. Pertinent laboratory results included a white blood cell count of 8.5 $\times 10^3/\mu L$ (reference range 4.5–10.8 $\times 10^3/\mu L$), hemoglobin 8.4 g/dL (reference range 13.5–17.5 g/dL), blood urea nitrogen 62 mg/dL (reference range 9–20 mg/dL), creatinine 4.5 mg/dL (reference range 0.6–1.2 mg/dL) and an estimated glomerular filtration rate of 12. Her baseline blood urea nitrogen and creatinine was 42 mg/dL and 3.0 mg/dL, respectively. Her glycated hemoglobin was

9.8%, indicating suboptimal diabetic control. Rapid HIV test was negative. Renal ultrasonogram revealed left hydronephrosis with doubtful renal calculi in the left ureter. Computed tomography of the abdomen without contrast was performed, which showed dilated left ureter with significant perinephric and periureteric stranding. There was left hydronephrosis and 5 mm calcification of the left base of the bladder suggestive of distal left ureteric stone. Culture of blood performed on the day of admission grew *S. cerevisiae*, an unusual pathogen to grow from blood. Her urine culture, stool culture and vaginal swab culture grew significant colony counts of *Saccharomyces cerevisiae*. Fungus isolated in the blood was sensitive to amphotericin, micafungin and fluconazole. Extensive review of her previous culture reports revealed that she never had any fungemia. The patient was treated with micafungin and her symptoms resolved. Repeat blood culture and urine culture were negative. She was then planned for a surgical removal of renal stone. But, the patient had relief of symptoms and repeat ultrasonogram revealed passage of stone with resolution of hydroureter. Her renal function returned back to her baseline. The patient was discharged home with arrangement for home intravenous antifungal treatment for a full course of 14 days.

Discussion

S. cerevisiae, also known as brewer's yeast or baker's yeast, is a common colonizer of human mucosal surfaces. Its role as a clinically important and invasive pathogen is not well known. There are very few case reports on invasive *S. cerevisiae*, and it causing acute pyelonephritis probably has never been reported. *S. cerevisiae* being a common colonizer and saprophytic contaminant, diagnosis may be often delayed and underestimated with potential lethal consequences.

It is a non-spore forming yeast, belonging to the family Saccharomycetaceae. Its nomenclature is derived from Latinized Greek meaning sugar mould, i.e. "Saccharo" meaning sugar and "myces" meaning mould or fungus. Cerevisiae comes from the latin word meaning "beer". *S. cerevisiae* is a single celled eukaryotic organism with a short generation time (doubling time 1.25–2 h) at 30°C (86°F) and can be easily cultured.[1] It has also been used as a probiotic. This organism is also used widely in the commercial setting for brewing beer and for providing CO_2 for underwater aquatic plants by "CO_2 injection by yeast technique." *S. cerevisiae* does not produce toxins that are harmful to humans or animals. However, it is capable of producing what are known as "killer toxins" that are fatal to other yeasts. *S. cerevisiae* is used in food and beverage preparation facilities to control the contamination of fermentation production areas by other kinds of yeasts. Heat-killed *Saccharomyces cerevisiae* (HKY) used as a vaccine protects mice against systemic aspergillosis and coccidioidomycosis.[2]

S. cerevisiae is a common colonizer of the human respiratory, gastrointestinal and urinary tracts and is generally considered as a benign organism. However, cases have been reported to cause invasive diseases in the setting of chronic underlying diseases like malignancy, HIV/AIDS or of bone marrow transplantation.[3,4] The evidences are in favor of the increased vulnerability of the host rather than the increased virulence of the pathogen in causing invasive disease. Pathogenic strains of *S. cerevisiae* exhibit the ability to grow at 42°C, produce proteinase and are capable of pseudohyphal growth. *S. cerevisiae* is often isolated with other forms of yeast, and it is rarely isolated as a single pathogen in yeast-induced vaginitis. One report on three cases of invasive *S. cerevisiae* infection causing pneumonia, liver abscess and sepsis indicates that pathogenic isolates of this yeast are capable of tissue invasion and dissemination. There are limited cases showing an association of this yeast and ingestion of vitamins and brewer's yeast. *S. boulardii*, used commonly as a biotherapeutic agent for chronic and recurrent diarrhea, is a strain of *S. cerevisiae* that has been implicated in a few cases of fungemia. *S. boulardii* analysis has revealed moderate

virulence levels when tested in murine models of systemic infection. Hence, caution is recommended when using this yeast to control this condition in severely ill or immunocompromised hosts. There are case reports of *S. cerevisiae* affecting newborns, where there is a mention of horizontal transmission of the disease from the primarily affected newborn that developed fungemia secondary to treatment with *S. boulardii*.

Although very rare, *S. cerevisiae* can cause invasive infections like pyelonephritis in immune-competent patients also, as in our case. There have been case descriptions of infection in patients with an indwelling catheter, prosthetic valves[5] and in bone marrow transplant patients.[6] In our patient, the presence of the renal stone could have caused obstruction of the urinary flow and hence had become a nidus for this organism, further confounded by a "semi-immunocompromised" state due to advanced chronic kidney disease and poorly controlled diabetes mellitus. With our patient having definite clinical and radiologic evidence of acute pyelonephritis, isolates of *S. cerevisiae* were found in literally all the bodily secretions; hence, proving that this normally benign organism could turn dangerously virulent in suitable conditions.

Diagnosing Saccharomyces infection is difficult as it is a normal flora in the human body. Isolating *S. cerevisiae* from the part of the body where it is normally colonized does not add much to clinical suspicion when the clinical symptoms and signs are subtle. However, the decision to attribute a causal role to *S. cerevisiae* is easier when the organism is isolated from a normally sterile body site, and the patient should be treated for an invasive fungal infection. There is no commercially available serological test for Saccharomyces. However, *S. cerevisiae* readily grows in blood cultures and on Sabouraud dextrose media.

Saccharomyces are susceptible to most antifungal drugs, including amphotericin B, 5-flucytosine, ketoconazole, fluconazole, itraconazole, voriconazole, posaconazole and isavuconazole,[7-9] although resistance to azoles have been reported.[10] However, most Saccharomyces isolates have higher minimum inhibitory concentrations (MIC) to most antifungal drugs than does *Candida albicans*. Patients would generally require a full two week course of medical treatment. Because the use of probiotic agents is widely prevalent in modern day practice, physicians need to be cognizant about this potential complication.

Conclusion

Even in people who have no prior exposure to probiotic agents, in the setting of impaired immune response, *S. cerevisiae* can cause invasive disease. In our patient, the fact that she had advanced chronic kidney disease and obstructive uropathy may have predisposed her to have invasive infection. Although this disease could be fatal, appropriate suspicion for this organism in susceptible persons, early recognition and treatment with anti-fungal medicines can cure the infection.

References

1. Fleet GH: Saccharomyces and related genera, Chapter 11, in: Food Spoilage Microorganisms. Boekhout T, Robert V. (eds), Behr's Verlag Hamburg, Germany, 2003. p. 306-335. .
2. Liu M, Clemons KV, Bigos M, Medovarska I, Brummer E, Stevens DA. Immune responses induced by heat killed Saccharomyces cerevisiae: A vaccine against fungal infection. Vaccine 2011;29:1745-53.
3. Sandhu G, Ranade A, Siddiqi S, Balderacchi JL. Essential thrombocythemia transforming into acute biphenotypic leukemia in a patient on hydroxyurea monotherapy.Ann Oncol 2009;20:1899-900.
4. Sandhu G, Dasgupta R, Ranade A, Baskin M. Pneumocystis zpneumonia in HIV-negative patient with no overt risk factors on presentation. EurRespir J 2010;35:927-9.
5. Nielsen H, Stenderup J, Bruun B. Fungemia with Saccharomycetaceae. Report of four cases and review of the literature. Scand J Infect Dis 1990;22:581-4.
6. Olver WJ, James SA, Lennard A, et al. Nosocomial transmission of Saccharomyces cerevisiae in bone marrow transplant patients. J Hosp Infect 2002;52:268-72.

7. Pfaller MA, Diekema DJ, Gibbs DL, et al. Results from the ARTEMIS DISK Global Antifungal Surveillance Study, 1997 to 2007: 10.5-year analysis of suceptibilities of non-candidal yeast species to fluconazole and voriconazole determined by CLSI standardized disk diffusion testing. J ClinMicrobiol 2009;47:117-23.
8. Thompson GR, Wiederhold NP, Sutton DA, Fothergill A, Patterson TE. In vitro activity of isavuconazole against Trichosporon, Rhodotorula, Geotrichum, Saccharomyces and Pichia species. J AntimicrobChemother 2009;64:79-83.
9. Sobel JD, Vazquez J, Lynch M, Meriwether C, Zervos MJ. Vaginitis due to Saccharomyces cerevisiae: Epidemiology, clinical aspects, and therapy. Clin Infect Dis 1993;16:93-9.
10. Salonen JH, Richardson MD, Gallacher K, ungal colonization of haematological patients receiving cytotoxic chemotherapy: Emergence of azole-resistant Saccharomyces cerevisiae. J Hosp Infect 2000;45:293-301.

Review
Allergy Asthma Immunol Res. 2013 November;5(6):348-356.
http://dx.doi.org/10.4168/aair.2013.5.6.348
pISSN 2092-7355 • eISSN 2092-7363

AAIR
Allergy, Asthma & Immunology Research

Diagnosis and Management of Grain-Induced Asthma

Santiago Quirce,[1]* Araceli Diaz-Perales[2]

[1]Department of Allergy, Hospital La Paz Institute for Health Research (IdiPAZ) and CIBER of Respiratory Diseases (CIBERES), Madrid, Spain
[2]Centro de Biotecnología y Genómica de Plantas (UPM-INIA), Pozuelo de Alarcón, Madrid, Spain

Grain-induced asthma is a frequent occupational allergic disease mainly caused by inhalation of cereal flour or powder. The main professions affected are bakers, confectioners, pastry factory workers, millers, farmers, and cereal handlers. This disorder is usually due to an IgE-mediated allergic response to inhalation of cereal flour proteins. The major causative allergens of grain-related asthma are proteins derived from wheat, rye and barley flour, although baking additives, such as fungal α-amylase are also important. This review deals with the current diagnosis and treatment of grain-induced asthma, emphasizing the role of cereal allergens as molecular tools to enhance diagnosis and management of this disorder. Asthma-like symptoms caused by endotoxin exposure among grain workers are beyond the scope of this review. Progress is being made in the characterization of grain and bakery allergens, particularly cereal-derived allergens, as well as in the standardization of allergy tests. Salt-soluble proteins (albumins plus globulins), particularly members of the α-amylase/trypsin inhibitor family, thioredoxins, peroxidase, lipid transfer protein and other soluble enzymes show the strongest IgE reactivities in wheat flour. In addition, prolamins (not extractable by salt solutions) have also been claimed as potential allergens. However, the large variability of IgE-binding patterns of cereal proteins among patients with grain-induced asthma, together with the great differences in the concentrations of potential allergens observed in commercial cereal extracts used for diagnosis, highlight the necessity to standardize and improve the diagnostic tools. Removal from exposure to the offending agents is the cornerstone of the management of grain-induced asthma. The availability of purified allergens should be very helpful for a more refined diagnosis, and new immunomodulatory treatments, including allergen immunotherapy and biological drugs, should aid in the management of patients with this disorder.

Key Words: Baker's asthma; cereals; wheat allergens; soya flour; fungal enzymes; allergen immunotherapy

INTRODUCTION

Asthma caused by allergy to proteins from cereal grains is one of the most common types of occupational asthma (OA) and its prevalence does not seem to be declining.[1] The main professions affected are: bakers, confectioners, pastry factory workers, millers, farmers, and cereal handlers. Although wheat is the most commonly involved cereal, other grains (e.g. rye, barley, rice) also play a role. In addition, flour from other sources (e.g. soya, lupin), pests, and several flour additives used in the baking industry to improve fermentation and elasticity of the dough, as well as to improve storage of the bread, may also give rise to IgE-mediated allergy.

EPIDEMIOLOGY

Work-related respiratory symptoms are highly prevalent among bakery workers, about 5%-10% suffer asthma and 15%-20% rhinitis. Baker's asthma is the most frequent type of OA in France.[2] Exposure to grain and flour dust is the second commonest reported cause of OA in the UK[3] and Norway.[4] The estimated annual incidence of cereal-induced asthma in the UK was 811 cases per million people employed over the period 1989-1997,[3] whereas in Norway, the incidence of OA among male and female bakers was 2.4 and 1 case per 1,000 person-years, respectively.[4] The incidence of baker's asthma among young bakers has been reported to range from 0.3 to 2.4 cases per 1,000 person-years.[5] In the last years an increasing number of asthma cases is being reported among supermarket bakery workers.[6]

In a prospective study carried out in Québec among apprentice bakers/pastry makers the incidence of work-related sensitization was 4.2% (per person-year) when the mean duration of follow up was lower than 4 years,[7] but it was 1.0 for a longer period (8 years) of follow up.[8]

Correspondence to: Santiago Quirce, MD, PhD, Department of Allergy, Hospital La Paz, Paseo de La Castellana, 261, 28046 Madrid, Spain.
Tel: +34-91-727-70-80; Fax: +34-91-727-70-50; E-mail: squirce@gmail.com
Received: January 2, 2013; Accepted: January 29, 2013
• There are no financial or other issues that might lead to conflict of interest.

PATHOGENESIS AND RISK FACTORS

This disorder has been classically considered a form of allergic asthma mediated by IgE antibodies specific to cereal flour antigens, mainly wheat, rye and barley,[9] but other cereal grains, such as rice, have also been implicated in cereal-induced asthma and rhinitis.[10,11] However, baking and food technology has notably progressed in the last decades and a vast array of biologic and chemical additives are now commonly used. Thus, it is not surprising that the list of causative agents of baker's asthma has been expanded with the demonstration that flours from different sources (soybean, buckwheat, and lupin), enzymes, egg proteins and organic contaminants such as storage mites, moulds and insects are also capable of causing IgE-mediated OA[12] (Table 1).

In a study in a Korean bakery, the overall prevalence of wheat sensitization was 5.9%, and it was confirmed that an IgE-mediated response is the major pathogenic mechanism for the induction of work-related symptoms in wheat-exposed workers, whereas wheat-specific IgG antibodies may represent current or previous exposure to wheat dust.[13]

Table 1. Allergenic sources described in grain/flour-induced asthma

	Scientific name	Reference
Cereal flour/dust		
Wheat	*Triticum aestivum*	9,12
Rye	*Secale cereale*	9,23
Barley	*Hordeum vulgare*	9,12
Rice	*Oryza sativa*	10,11,65
Fungal enzymes		
Alpha-amylase	*Aspergillus oryzae* (Asp o 21)	76,78
Hemicellulase/cellulase	*Aspergillus niger*	77,78
Glucoamylase	*Aspergillus niger*	79
Beta-xyloxidase	*Aspergillus niger* (Asp n 14)	77
Xylanase	*Aspergillus niger*	80
Legumes		
Soya flour	*Glycine max*	81,82
Soybean lecithin	*Glycine max*	83
Lupin flour	*Lupinus albus*	84
Baker's yeast	*Saccharomyces cerevisiae*	85
Other ingredients		
Buckwheat flour	*Fagopyrum esculentum*	86
Sunflower seeds	*Helianthus annuus*	87
Egg white proteins	Gal d 1, Gal d 2, Gal d 3, Gal d 4	88
Storage mites		89,90
	Lepidoglyphus destructor	
	Acarus siro	
	Tyrophagus putrescentiae	
Arthropods		
Grain weevil	*Sitophilus granarius*	91
Flour moths	*Ephestia spp. and Eurigaster* spp.	92
Spider	*Holocnemus pluchei*	93
Molds		94
	Aspergillus fumigatus	
	Alternaria alternata	

Moreover, workers handling cereal or vegetable seeds are at risk of exposure to high levels of endotoxin containing seed dust. Occupational exposure to inhalable agricultural seed dust can induce inflammatory responses, and is a potential cause of organic dust toxic syndrome (ODTS), which typically presents with asthma-like symptoms, and is an important differential diagnosis of OA.[14] Moreover, exposure to flour allergens and endotoxins interact to induce allergic responses and respiratory symptoms. Grain dust-related asthma symptoms caused by exposure to endotoxins, which have been reported among agricultural and grain elevator workers, are not addressed in this review.

Houba et al.[15] investigated the relationship between allergen exposure and IgE sensitization to wheat flour among bakery workers. A strong and positive association was found between wheat flour allergen exposure and wheat flour sensitization, for both atopic and non-atopic workers, and this relationship was steepest within the group of atopic bakers. This means that the likelihood of sensitization increased with increasing wheat allergen concentration in the workplace air, and the risk was higher for atopic subjects.

Several studies have shown that the level of exposure and atopy are the main risk factors for developing sensitization and work-related symptoms among subjects exposed to cereal flour. Age, gender, and smoking habits do not seem to be associated with sensitization or work-related respiratory symptoms.[12] A study carried out in Belgium compared bakery workers with a nonexposed work population and showed that atopy and sensitization to bakery allergens were independent and additional risk factors for work-related symptoms.[16]

Genetic factors seem to be also important in the development of work-related respiratory symptoms and sensitization to wheat flour in bakery workers. In this regard, Cho et al.[17] carried out a study in Korean bakery workers, and they found that Toll-like receptor 4 (TLR4) gene polymorphisms may be involved in allergic sensitization to wheat flour as well as endotoxin-induced respiratory symptoms in endotoxin-allergen-exposed workers and that carriers of TLR4 variants are less affected by environmental exposure. In a study in Korean bakery workers, Hur et al.[18] reported that the genetic polymorphisms of β2-adrenergic receptors (ADRB2) may contribute to the development of work-related symptoms in workers exposed to wheat flour, which can lead to baker's asthma.

DIAGNOSIS

The diagnosis of grain-induced asthma depends on a consis-

tent history of work-related asthma symptoms, assessment of IgE-mediated sensitization (by means of skin prick tests or *in vitro* tests) to cereal proteins or other bakery allergens, and evidence of variability in lung function. The diagnosis can be confirmed by specific inhalation challenge (SIC) with bakery allergens.

In epidemiological studies the diagnosis of OA is usually performed using a combination of symptoms and bronchial hyperresponsiveness (BHR) to pharmacological agents (usually methacholine or histamine), while in the clinical setting the 'gold standard' for the diagnosis of OA is the SIC test in the laboratory. However, a step-by-step approach has also been proposed for cross-sectional epidemiological studies in workers exposed to grain and/or flour dust.[19] BHR was observed in a small percentage of subjects with asthma-like symptoms and/or low FEV1, and a positive response to SIC to wheat flour was observed in a subgroup of subjects with BHR, stressing the need to perform SIC to confirm the diagnosis of flour-induced OA.[19]

Rhinitis, with or without conjunctivitis, is commonly associated with flour-induced asthma, and should also be considered in the diagnosis and management.

Skin prick tests

The frequency of sensitization to wheat flour by SPT among cereal workers in epidemiological studies varies from 5% to 15%.[12] Skin reactivity is related to the quality, potency and standardization of allergen extracts, which are often poorly defined for cereal and other occupational allergens. Sander et al.[20] carried out a study to compare different wheat and rye flour extracts used for skin testing, and related the results to the outcome of SIC as gold standard. Wheat and rye flour extracts for SPT from three companies differed in protein concentrations and composition, resulting in a wide difference in SPT results. Sensitivity of SPTs was between 40% and 67%, specificity was between 86% and 100%, the positive predictive value (PPV) ranged from 81% to 100% and the negative predictive value (NPV) from 44% to 70%.[20]

van Kampen et al.[21] carried out a multicenter study in which SPT were performed with wheat and rye flour extracts from 4 producers in 125 symptomatic bakers. Comparisons between SPT results of different extracts were made with flour-specific IgE. The optimal cut-off level for all SPT solutions was a weal size of $\geq$1.5 mm. Again, a wide variability of SPT wheat and rye flour extracts from different producers was found. These data indicate that improvement and standardization of SPT solutions used for the diagnosis of cereal-induced asthma are needed.

Specific IgE measurements

The sensitivity of specific IgE measurements (by either ImmunoCAP or ELISA) has been shown to be higher than SPT with commercial cereal (wheat and rye) extracts.[20] The sensitivity of specific IgE to wheat and rye flour was 83% and 72%, respectively, whereas the specificity was 59% and 81%.

Specific inhalation challenge

SIC is still considered the gold standard for the diagnosis of baker's asthma.[22] Despite the broad allergenic cross-reactivity between wheat and rye flour, some patients may have a negative SIC with wheat flour and a positive reaction to SIC with rye flour,[23] indicating that SIC should be performed with flour from different cereals.

van Kampen et al.[24] evaluated the relevance of flour-specific serum IgE and SPT in the diagnosis of baker's asthma and for the purpose of defining flour-specific IgE concentrations and wheal sizes that make it possible to predict the outcome of SIC. The results of the challenge with wheat flour were positive for 37 bakers, while 63 had positive results with rye flour. Depending on the flour-specific IgE concentrations (wheal size), positive predictive value (PPV) was 74%-100% for wheat and 82%-100% for rye flour, respectively. The minimal cutoff values with a PPV of 100% were 2.32 kU/L (wheal size 5.0 mm) for wheat flour, and 9.64 kU/L (wheal size 4.5 mm) for rye flour. Thus, high concentrations of flour-specific IgE and clear SPT results in symptomatic bakers are good predictors of a positive challenge test result.

These observations suggest that SIC with cereal flours can be avoided in strongly sensitized bakers. In fact, a systematic literature review showed that in workers with suspected OA caused by high-molecular-weight agents, a positive SPT result and BHR to methacholine correlates with SIC (high specificity, moderate sensitivity).[25]

The main determinant of a positive SIC to an allergen in patients with baker's asthma is the degree of sensitization to that allergen as determined by skin reactivity, modulated to a lesser extent by non-specific BHR.[26]

On the other hand, in baker's with persistent cough and a negative asthmatic response to the SIC, a diagnosis of non-asthmatic eosinophilic bronchitis should be considered. Monitoring of airway inflammation by non-invasive methods (induced sputum and/or exhaled nitric oxide) is necessary to confirm the diagnosis.[27,28]

WHEAT GRAIN PROTEINS AND INHALANT ALLERGENS

Protein represents about 10%-15% (dry weight) of wheat grain. It can be classified in four different fractions based on sequential extraction in a series of solvents.[29] Salt-soluble fractions, named albumins and globulins, include only 15%-20% of the total protein, whereas most protein components, designated prolamins (gliadins plus glutenins), are not extracted by salt solutions. Gliadins are monomeric proteins, and are grouped into three types, designated α/β-, γ- and ω-gliadins, according to their electrophoretic mobility at low pH and biochemical characteristics.[30] Glutenins form polymers maintained by inter-

chain disulphide bridges, and are classified into high molecular weight (HMW) and low molecular weight (LMW) glutenin subunits.[30]

The sequential extraction procedure does not render clear-cut preparations, as expected. Thus, cross-contamination among protein fractions can occur (i.e. some salt-soluble proteins, such as α-amylase inhibitor subunits, can residually appear in the glutenin fraction).[31] Nevertheless, the peculiar extractability properties of wheat grain proteins lead to several constrain of commercial diagnostic products. Thus, wheat ImmunoCAP contains preponderantly salt-soluble proteins, and has to be complemented with glutenin ImmunoCAP, and/or ω-5 gliadin ImmunoCAP, for proper diagnosis of same patients.[32]

A high degree of heterogeneity of recognized allergens between groups with different clinical profiles (food allergy, wheat-dependent exercise induced anaphylaxis –WDEIA–, baker's asthma), as well as within each group, has been reported.[33] However, mainly salt-soluble proteins seem to be associated with baker's asthma.

The main allergens implicated in cereal-induced asthma are: the α-amylase/trypsin inhibitor family, lipid transfer protein (LTP), peroxidase, thioredoxin, serine proteinase inhibitor, thaumatin-like protein and some prolamins.

Besides the above mentioned allergens, additional IgE-binding proteins, such as acyl-CoA oxidase, fructose-bisphosphate aldolase, triosephosphate isomerise, glycerinaldehyde-3-phosphate dehydrogenase, and serpin, has been located by a proteomic approach and IgE-immunodetection.[34,35] On the other hand, β-amylase (from barley flour)[36] and Tri a Bd 27K, a member of the γ-interferon-inducible thiol reductases,[37] have been reported as putative allergens. An association between baker's asthma and allergy to kiwifruit[38] has been described, suggesting that wheat thiol-proteases homologous to kiwi Act d 1 can be responsible for wheat-kiwi cross-reactivity.

Recently, Pahr et al.,[39] using a cDNA library, have reported the molecular characterization, recombinant expression and purification of five wheat allergens involved in respiratory allergy: 1-Cys-peroxiredoxin, dehydrin, a thioredoxin h isoform, glutathione transferase, and profilin.

The cereal α-amylase/trypsin inhibitor family

The α-amylase/trypsin inhibitor family is regarded as the major type of wheat allergens responsible for cereal asthma.[29,40] This family comprises a large proportion of the salt-soluble proteins from wheat flour, including the so-called CM proteins, which besides salt-solutions, can be also extracted with chloroform/methanol mixtures.[41]

The cereal α-amylase/trypsin inhibitor subunits are 12-16 kDa polypeptides with 4-5 intrachain disulphide bridges essential for their inhibitory activity.[41] Members of the inhibitor family are restricted to the seed storage tissue (endosperm) and seem to have a common fold.[41,42]

The inhibitor subunits are encoded by a multigene family in wheat, rye and barley. Thus, up to 12 different subunits have been characterized in a single bread wheat (*Triticum aestivum*) cultivar, most of them with IgE-binding capacity (see below).[40,41] Amino acid sequence identity between members of the family ranges from around 30% to 95%.

Three types of α-amylase inhibitors have been identified in wheat flour based on their degree of aggregation: monomeric (1 subunit), homodimeric (2 identical subunits) and heterotetrameric (3 different subunits, one of them in two copies).[40,41,43]

Activity towards heterologous α-amylases from insects, mites, mammals and/or bacteria, but not against the endogenous α-amylases present in the cereal kernel, has been described for the wheat inhibitors.[40,41] Their role in plant defence is supported by the negative effects on the amylase activity of coleopteran and lepidopteran pests, mainly predators of stored cereal grains.[44,45]

Extensive data reported by different groups, mainly based on 1- and 2-dimensional immunoblotting of wheat flour salt-soluble proteins, allowed to identify several 12-16 kDa members of the α-amylase/trypsin inhibitor family as major IgE-binding proteins in sera from wheat-induced asthmatic patients.[34-36,46-51] The *in vitro* analysis of purified inhibitor subunits fully confirmed their IgE-binding capacity.[47,49,51,52]

Biological activity of allergenic wheat α-amylase inhibitors was indicated by the induction of histamine release from peripheral basophils by wheat flour fractions enriched in salt-soluble 14 kDa electrophoretic bands,[53] and by the stimulation of leukotriene release from peripheral leucocytes by both purified WMAI-1 and WDAI-2 allergens.[51]

Armentia et al.[54] carried out SPT with 6 purified inhibitor subunits from wheat flour, which allowed evaluating the *in vivo* reactivity of representative members of this allergen family. Most asthmatic patients (87%) sensitized to wheat flour reacted to a protein preparation enriched in inhibitor subunits, as well as (80%) to at least one isolated wheat subunit. However, positive responses to purified inhibitor allergens so far assayed varied from 16% to 45%, being a glycosylated tetrameric inhibitor subunit (gWTAI-CM16) the highest reactive protein.

Homology among inhibitor subunits from wheat, rye and barley partially accounts for cross-reactivity between flours from these cereals.[40,48,50] Allergens belonging to the α-amylase inhibitor family and associated with baker's asthma have been isolated from rye[55,56] and barley[54,57] flour, and their reactivity confirmed by *in vivo* SPT.[54,56] Interestingly, three of the rye inhibitors tested, namely Sec c 1, RDAI-1 and RDAI-3, provoked positive SPT responses in more than 50% of 21 patients with baker's asthma induced by rye flour.

Peroxidase

Sanchez-Monge et al.[58] isolated a prominent IgE-binding protein of 36 kDa from diploid (*Triticum monococcum*) wheat flour. The protein, which is also present in tetraploid (pasta) and hexa-

ploid (bread) wheat, was identified as a seed-specific peroxidase harbouring N-linked complex glycans (CCDs). Sera from 6 out of 10 patients with baker's asthma displayed *in vitro* (dot-blot) reaction to the purified allergen. The biochemical characteristics of the peroxidase were latter confirmed by Yamashita et al.,[59] who suggested IgE-binding to the glycan moiety.

Thioredoxin

Thioredoxins are 12-14 kDa ubiquitous regulatory proteins that reduce intrachain disulphide bridges of target proteins, such as the wheat storage prolamins (gliadins and glutenins), thus enhancing the mobilization of these storage proteins in germinating wheat seeds.[60] Wheat thioredoxin, named Tri a 25, has been located as a novel allergen related to cereal-induced asthma by screening of a wheat cDNA phage display library with sera from patients suffering this occupational disease.[61]

Both Tri a 25 and its homologous (74% of amino acid sequence identity) maize thioredoxin Zea m 25 have been produced in *E. coli* as recombinant proteins, and tested against 17 sera from patients with baker's asthma.[61]

Non-specific lipid transfer protein (LTP)

Plant LTPs constitute a panallergen family of 9 kDa basic polypeptides, which show a 3D-fold characterized by a compact domain composed of 4 α-helices strongly linked by a network of 4 conserved disulphide bridges.[62] A main *in vivo* function of these proteins seems to be their involvement in plant defence mechanisms against phytopathogens (bacteria and fungi), this leading to their classification as pathogenesis-related (PR) proteins (PR-14 family).[62]

Palacin et al.[63] have characterized wheat flour LTP, named wheat Tri a 14, as a major allergen associated with baker's asthma. Specific IgE to this wheat flour LTP was detected in 60% of sera from 40 Spanish patients with baker's asthma, and *in vivo* reactivity (positive SPT response) found in 15 (62%) out of 24 of these patients. Furthermore, recombinant Tri a 14 has been produced in *Pichia pastoris*, and its physicochemical properties, heat and proteolytic resistance, and IgE-binding capacity shown almost equivalent to those of its natural counterpart.[64] These characteristics, together with its biological potency,[64] makes rTri a 14 a helpful tool for the diagnosis of cereal-induced asthma. Moreover, LTP has also been implicated in rhinitis and asthma caused by rice inhalation.[65]

Serine proteinase inhibitor

Constantin et al.[66] identified in 2008 a serine proteinase inhibitor as a novel allergen in baker's asthma, by screening of a cDNA library from wheat seeds with serum IgE from asthmatic patients. The allergen is a 9.9 kDa protein, usually forming 40 kDa tetramers, which represent a new member of the potato inhibitor I family. The inhibitor is mainly expressed in mature seeds, being accumulated in the starchy endosperm and the aleuron layer. It is probably involved in plant defence and belongs to the pathogenesis-related (PR) protein-6 family.

The recombinant form of this allergen produced in *E. coli*, but not the natural one, has been assayed against sera from baker's asthma patients.[66] The recombinant allergen reacted with specific IgE from 14%[66] and 27%[67] sera from Spanish baker's asthma subjects, when tested in dot blotted or micro-arrayed samples, respectively. In contrast, the inhibitor was not recognized by sera from patients suffering wheat food or grass pollen allergy.[66,67] Biological activity of the recombinant allergen was ascertained by 3 out of 3 positive basophil histamine release assays.[66]

Despite around 50% amino acid sequence identity, no relevant cross-reactivity was found with homologous inhibitors from maize and rice.[66]

Thaumatin-like protein

Thaumatin-like proteins (TLPs) is the latest salt-soluble protein family from wheat flour that has been associated to baker's respiratory allergy by Lehto et al.[68] in 2010. Most TLPs have molecular masses ranging from 21 to 26 kDa, and 16 conserved cysteine residues forming 8 disulphide bridges that are responsible for a compact 3D-structure and resistance to low pH conditions, proteolysis and heat treatment.[69] TLP antifungal activity supports the role of these proteins in plant defence against fungal pathogens and their assignment to form family 5 of the pathogenesis-related (PR) proteins.[69]

Purified wheat TLP induces positive SPT responses in 30% to 45% Finnish patients with baker's asthma when tested at 50 μg/mL and 500 μg/mL, respectively.[68]

Prolamins: gliadins and glutenins

Several of the major water/salt-insoluble wheat flour proteins (prolamins) also appear to be implicated in baker's asthma. Several studies[70-72] have demonstrated IgE-binding in the prolamin fraction, including by α-, β-, γ- and ω-gliadins, and LMW-glutenin subunits.[71] However, very few studies have evaluated the allergenicity of purified (recombinant) prolamins.[73,74] Bittner et al.[74] expressed in *E. coli* a cDNA encoding an αβ-gliadin of 20 kDa, and isolated the corresponding recombinant prolamin. ELISA screening of 153 sera of bakers with OA using the recombinant αβ-gliadin, detected specific IgE in 12% of the sera tested. Of the asthmatic bakers, 33% showed sensitization to natural total gliadin.

OTHER ALLERGENS ASSOCIATED WITH GRAIN INDUCED ASTHMA

In addition to cereal proteins, a wide array of components, mostly additives used to improve wheat flour quality for baking, such as fungal enzymes (mainly α-amylase from *Aspergillus oryzae*, termed Asp o 21), have been also associated with baker's asthma.[75]

Flour improvers

Starch-cleaving enzymes are well known additives in processing of fermented dough products and are widely used in the baking industry to hasten and economize the bread-making process. By hydrolyzing starch molecules to fermentable sugars, these enzymes continuously generate a substrate that can be used by yeast.

Baur et al.[76] reported in 1986 the first description of allergy to α-amylase from *Aspergillus oryzae* in baker's asthma. Fungal α-amylase is an endoglycosidase which catalyses the hydrolysis of internal alpha-1, 4-glycosidic linkages in various polysaccharides yielding a mixture of maltose and glucose. This enzyme is added to flour (in amounts of milligrams per kg flour) to compensate for the low natural content of amylases of cereal flour and so enhance carbohydrate fermentation by yeast. These authors found that 12 out of 35 (34%) bakers with asthma had developed IgE-mediated allergy to α-amylase.[76] More recent studies have shown that 23% of German bakers suffering from workplace-related rhinitis and/or asthma were sensitized to fungal α-amylase.[77]

In addition, other fungal enzymes used as baking additives such as hemicellulase, cellulase, xylanase, and glucoamylase (or amyloglucosidase) from *Aspergillus niger* have been shown to cause IgE-mediated sensitization by means of *in vitro* and *in vivo* tests.[77-80] The frequency of serum specific IgE to glucoamylase among symptomatic bakers varies from 5% to 8%, indicating that this enzyme may be a relevant allergen in the baking industry.[79]

Soya flour is used as a baking additive for the emulsifying properties of lecithin and the whitening effect of lipoxygenase. The presence of specific IgE antibodies recognizing lipoxygenase and/or soybean trypsin inhibitor have been demonstrated in symptomatic bakers,[75,81,82] and soybean lecithin has also been reported as a causative agent of OA.[83] More recently, cases of rhinitis and asthma caused by lupin flour have been reported but their impact in the bakery sector is not yet established.[84]

Other bakery ingredients that have been implicated in isolated cases of baker's asthma are baker's yeast (*Saccharomyces cerevisiae*),[85] buckwheat,[86] sunflower seed,[87] and egg proteins.[88]

Grain dust contaminants and pests

Storage mite (*Lepidoglyphus destructor, Acarus siro and Tyrophagus putrescentiae*) have been frequently implicated in cereal asthma but their causative role has not been demonstrated beyond doubt.[89,90] Other insects,[91,92] spiders[93] and fungi[94] that may contaminate grain and/or flour have been incriminated in respiratory allergy, but their role appears to be extremely limited.

MANAGEMENT

As in other types of immunologic OA, early diagnosis and early avoidance of further exposure are the cornerstones of management for patients with grain-induced asthma.[95] Whenever feasible the patient should be relocated to a job category without exposure. Pharmacological treatment of OA should comply with published asthma guidelines.

Allergen-specific immunotherapy (SIT) and other biological treatments, such as anti-IgE monoclonal antibodies (omalizumab), also may play a role in management.

Armentia et al.[96] published the results of the first double-blind placebo-controlled study of SIT with cereal flour in baker's asthma in 1990. Twenty patients were treated with an aqueous wheat flour extract (ALK-Abelló, Madrid, Spain) and 10 with placebo for 10 and 20 months. After SIT, the active group showed a significant decrease in skin sensitivity and also in bronchial hyperresponsiveness to methacholine. Specific IgE to wheat flour decreased only in patients who were treated with SIT for 20 months. Patients in the active group also reported a significant subjective improvement, whereas patients in the placebo group showed no changes in skin sensitivity or BHR to methacholine.

Case reports[97] and retrospective studies[98] have also shown efficacy of wheat flour SIT in baker's asthma. An observational cross-sectional retrospective study was performed on 41 sensitised bakers[98] who underwent subcutaneous SIT with wheat flour extract (Lofarma Allergeni, Milan, Italy) for 4 or more years, without avoiding their work activity. The outcome was investigated after 5 or 10 years. Thirty-four subjects out of 41 were still at work with an acceptable quality of life and a normal working activity, mainly in their small enterprises. In the subgroup of 19 patients treated in the past, several bakers still at work had stopped SIT even from 4-10 years. In the subgroup of 15 patients, still in treatment, symptoms and drug use during the work activity resulted to be reduced or absent in the majority of cases. The authors suggested that SIT with wheat flour may allow relocation in many of cases of baker's asthma and may be associated with other environmental interventions in the workplaces.[98]

Omalizumab has been shown to have clinical benefit in patients with uncontrolled severe baker's asthma.[99,100]

ACKNOWELDGMENTS

In memoriam of Prof. Gabriel Salcedo, for his outstanding contribution, leadership and teaching on biochemistry and allergen characterization, specifically on plant and cereal allergens.

REFERENCES

1. Malo JL, Chan-Yeung M. Agents causing occupational asthma. J Allergy Clin Immunol 2009;123:545-50.
2. Ameille J, Pauli G, Calastreng-Crinquand A, Vervloët D, Iwatsubo Y, Popin E, Bayeux-Dunglas MC, Kopferschmitt-Kubler MC; Observatoire National des Asthmes Professionnels. Reported incidence

of occupational asthma in France, 1996-99: the ONAP programme. Occup Environ Med 2003;60:136-41.
3. McDonald JC, Keynes HL, Meredith SK. Reported incidence of occupational asthma in the United Kingdom, 1989-97. Occup Environ Med 2000;57:823-9.
4. Leira HL, Bratt U, Slåstad S. Notified cases of occupational asthma in Norway: exposure and consequences for health and income. Am J Ind Med 2005;48:359-64.
5. Rémen T, Coevoet V, Acouetey DS, Guéant JL, Guéant-Rodriguez RM, Paris C, Zmirou-Navier D. Early incidence of occupational asthma among young bakers, pastry-makers and hairdressers: design of a retrospective cohort study. BMC Public Health 2010;10:206.
6. Baatjies R, Lopata AL, Sander I, Raulf-Heimsoth M, Bateman ED, Meijster T, Heederik D, Robins TG, Jeebhay MF. Determinants of asthma phenotypes in supermarket bakery workers. Eur Respir J 2009;34:825-33.
7. Gautrin D, Ghezzo H, Infante-Rivard C, Malo JL. Incidence and determinants of IgE-mediated sensitization in apprentices. A prospective study. Am J Respir Crit Care Med 2000;162:1222-8.
8. Gautrin D, Ghezzo H, Infante-Rivard C, Magnan M, L'archevêque J, Suarthana E, Malo JL. Long-term outcomes in a prospective cohort of apprentices exposed to high-molecular-weight agents. Am J Respir Crit Care Med 2008;177:871-9.
9. Brant A. Baker's asthma. Curr Opin Allergy Clin Immunol 2007;7:152-5.
10. Kim JH, Choi GS, Kim JE, Ye YM, Park HS. Three cases of rice-induced occupational asthma. Ann Allergy Asthma Immunol 2010;104:353-4.
11. Kim JH, Kim JE, Choi GS, Hwang EK, An S, Ye YM, Park HS. A case of occupational rhinitis caused by rice powder in the grain industry. Allergy Asthma Immunol Res 2010;2:141-3.
12. Houba R, Doekes G, Heederik D. Occupational respiratory allergy in bakery workers: a review of the literature. Am J Ind Med 1998;34:529-46.
13. Hur GY, Koh DH, Kim HA, Park HJ, Ye YM, Kim KS, Park HS. Prevalence of work-related symptoms and serum-specific antibodies to wheat flour in exposed workers in the bakery industry. Respir Med 2008;102:548-55.
14. Smit LA, Wouters IM, Hobo MM, Eduard W, Doekes G, Heederik D. Agricultural seed dust as a potential cause of organic dust toxic syndrome. Occup Environ Med 2006;63:59-67.
15. Houba R, Heederik D, Doekes G. Wheat sensitization and work-related symptoms in the baking industry are preventable. An epidemiologic study. Am J Respir Crit Care Med 1998;158:1499-503.
16. Droste J, Myny K, Van Sprundel M, Kusters E, Bulat P, Braeckman L, Vermeire P, Vanhoorne M. Allergic sensitization, symptoms, and lung function among bakery workers as compared with a nonexposed work population. J Occup Environ Med 2003;45:648-55.
17. Cho HJ, Kim SH, Kim JH, Choi H, Son JK, Hur GY, Park HS. Effect of Toll-like receptor 4 gene polymorphisms on work-related respiratory symptoms and sensitization to wheat flour in bakery workers. Ann Allergy Asthma Immunol 2011;107:57-64.
18. Hur GY, Park HJ, Lee HY, Koh DH, Lee BJ, Choi GS, Kim SH, Ye YM, Park HS. Association of beta(2)-adrenergic receptor polymorphism with work-related symptoms in workers exposed to wheat flour. Yonsei Med J 2011;52:488-94.
19. Talini D, Benvenuti A, Carrara M, Vaghetti E, Martin LB, Paggiaro PL. Diagnosis of flour-induced occupational asthma in a cross-sectional study. Respir Med 2002;96:236-43.
20. Sander I, Merget R, Degens PO, Goldscheid N, Brüning T, Raulf-Heimsoth M. Comparison of wheat and rye flour skin prick test solutions for diagnosis of baker's asthma. Allergy 2004;59:95-8.
21. van Kampen V, Merget R, Rabstein S, Sander I, Bruening T, Broding HC, Keller C, Muesken H, Overlack A, Schultze-Werninghaus G, Walusiak J, Raulf-Heimsoth M. Comparison of wheat and rye flour solutions for skin prick testing: a multi-centre study (Stad 1). Clin Exp Allergy 2009;39:1896-902.
22. De Zotti R, Bovenzi M, Negro C, Cirla A, Innocenti A, Lorusso A, Mariano A, Paggiaro PL, Talini D, Pisati G, Romano C, Sulotto F. Specific inhalation challenge with wheat flour in workers with suspected baker's asthma. Int Arch Occup Environ Health 1999;72:335-7.
23. Letrán A, Palacín A, Barranco P, Salcedo G, Pascual C, Quirce S. Rye flour allergens: an emerging role in baker's asthma. Am J Ind Med 2008;51:324-8.
24. van Kampen V, Rabstein S, Sander I, Merget R, Brüning T, Broding HC, Keller C, Müsken H, Overlack A, Schultze-Werninghaus G, Walusiak J, Raulf-Heimsoth M. Prediction of challenge test results by flour-specific IgE and skin prick test in symptomatic bakers. Allergy 2008;63:897-902.
25. Beach J, Rowe BH, Blitz S, Crumley E, Hooton N, Russell K, Spooner C, Klassen T. Diagnosis and management of work-related asthma. Evid Rep Technol Assess (Summ) 2005;(129):1-8.
26. Quirce S, Fernández-Nieto M, Escudero C, Cuesta J, de Las Heras M, Sastre J. Bronchial responsiveness to bakery-derived allergens is strongly dependent on specific skin sensitivity. Allergy 2006;61:1202-8.
27. Quirce S, Lemière C, de Blay F, del Pozo V, Gerth Van Wijk R, Maestrelli P, Pauli G, Pignatti P, Raulf-Heimsoth M, Sastre J, Storaas T, Moscato G. Noninvasive methods for assessment of airway inflammation in occupational settings. Allergy 2010;65:445-58.
28. Barranco P, Fernández-Nieto M, del Pozo V, Sastre B, Larco JI, Quirce S. Nonasthmatic eosinophilic bronchitis in a baker caused by fungal alpha-amylase and wheat flour. J Investig Allergol Clin Immunol 2008;18:494-5.
29. Tatham AS, Shewry PR. Allergens to wheat and related cereals. Clin Exp Allergy 2008;38:1712-26.
30. Shewry PR, Tatham AS, Halford NG. The prolamins of the Triticeae. In: Shewry PR, Casey R, editors. Seed proteins. Dordrecht: Kluwer Academic Publishers; 1999. 35-78.
31. Pastorello EA, Farioli L, Conti A, Pravettoni V, Bonomi S, Iametti S, Fortunato D, Scibilia J, Bindslev-Jensen C, Ballmer-Weber B, Robino AM, Ortolani C. Wheat IgE-mediated food allergy in European patients: alpha-amylase inhibitors, lipid transfer proteins and low-molecular-weight glutenins. Allergenic molecules recognized by double-blind, placebo-controlled food challenge. Int Arch Allergy Immunol 2007;144:10-22.
32. Salcedo G, Quirce S, Diaz-Perales A. Wheat allergens associated with Baker's asthma. J Investig Allergol Clin Immunol 2011;21:81-92.
33. Sander I, Raulf-Heimsoth M, Düser M, Flagge A, Czuppon AB, Baur X. Differentiation between cosensitization and cross-reactivity in wheat flour and grass pollen-sensitized subjects. Int Arch Allergy Immunol 1997;112:378-85.
34. Weiss W, Huber G, Engel KH, Pethran A, Dunn MJ, Gooley AA, Görg A. Identification and characterization of wheat grain albumin/globulin allergens. Electrophoresis 1997;18:826-33.
35. Sander I, Flagge A, Merget R, Halder TM, Meyer HE, Baur X. Iden-

tification of wheat flour allergens by means of 2-dimensional immunoblotting. J Allergy Clin Immunol 2001;107:907-13.
36. Sandiford CP, Tee RD, Taylor AJ. The role of cereal and fungal amylases in cereal flour hypersensitivity. Clin Exp Allergy 1994;24:549-57.
37. Kimoto M, Suzuki M, Komiyama N, Kunimoto A, Yamashita H, Hiemori M, Takahashi K, Tsuji H. Isolation and molecular cloning of a major wheat allergen, Tri a Bd 27K. Biosci Biotechnol Biochem 2009;73:85-92.
38. Palacin A, Quirce S, Sánchez-Monge R, Fernández-Nieto M, Varela J, Sastre J, Salcedo G. Allergy to kiwi in patients with baker's asthma: identification of potential cross-reactive allergens. Ann Allergy Asthma Immunol 2008;101:200-5.
39. Pahr S, Constantin C, Mari A, Scheiblhofer S, Thalhamer J, Ebner C, Vrtala S, Mittermann I, Valenta R. Molecular characterization of wheat allergens specifically recognized by patients suffering from wheat-induced respiratory allergy. Clin Exp Allergy 2012;42:597-609.
40. Salcedo G, Sanchez-Monge R, Garcia-Casado G, Armentia A, Gomez L, Barber D. The cereal α-amylase/trypsin inhibitor family associated with baker's asthma and food allergy. In: Mills ENC, Shewry PR, editors. Plant food allergens. Oxford: Blackwell Science; 2004. 70-86.
41. Carbonero P, Salcedo G, Sanchez-Monge R, Garcia-Maroto F, Royo J, Gomez L, Mena M, Medina J, Diaz I. A multigene family from cereals which encodes inhibitors of trypsin and heterologous α-amylases. In: Aviles FX, editor. Innovations in proteases and their inhibitors. Berlin: Walter de Gruyter; 1993. 333-48.
42. Oda Y, Matsunaga T, Fukuyama K, Miyazaki T, Morimoto T. Tertiary and quaternary structures of 0.19 alpha-amylase inhibitor from wheat kernel determined by X-ray analysis at 2.06 A resolution. Biochemistry 1997;36:13503-11.
43. Gomez L, Sanchez-Monge R, Garcia-Olmedo F, Salcedo G. Wheat tetrameric inhibitors of insect alpha-amylases: Alloploid heterosis at the molecular level. Proc Natl Acad Sci U S A 1989;86:3242-6.
44. Gutierrez C, Sanchez-Monge R, Gomez L, Ruiz-Tapiador M, Castañera P, Salcedo G. α-Amylase activities of agricultural insect pests are specifically affected by different inhibitor preparations from wheat and barley endosperms. Plant Sci 1990;72:37-44.
45. Gutierrez C, Garcia-Casado G, Sanchez-Monge R, Gomez L, Castañera P, Salcedo G. The three inhibitor types from wheat endosperma are differentially active against α-amylases of Lepidoptera pests. Entomol Exp Appl 1993;66:47-52.
46. Pfeil T, Schwabl U, Ulmer WT, König W. Western blot analysis of water-soluble wheat flour (Triticum vulgaris) allergens. Int Arch Allergy Appl Immunol 1990;91:224-31.
47. Gómez L, Martín E, Hernández D, Sánchez-Monge R, Barber D, del Pozo V, de Andrés B, Armentia A, Lahoz C, Salcedo G, Palomino P. Members of the alpha-amylase inhibitors family from wheat endosperm are major allergens associated with baker's asthma. FEBS Lett 1990;261:85-8.
48. Fränken J, Stephan U, Neuber K, Bujanowski-Weber J, Ulmer WT, König W. Characterization of allergenic components of rye and wheat flour (Secale, Triticum vulgaris) by western blot with sera of bakers: their effects on CD23 expression. Int Arch Allergy Appl Immunol 1991;96:76-83.
49. Sanchez-Monge R, Gomez L, Barber D, Lopez-Otin C, Armentia A, Salcedo G. Wheat and barley allergens associated with baker's asthma. Glycosylated subunits of the alpha-amylase-inhibitor family have enhanced IgE-binding capacity. Biochem J 1992;281:401-5.
50. Sandiford CP, Tee RD, Newman-Taylor AJ. Identification of cross-reacting wheat, rye, barley and soya flour allergens using sera from individuals with wheat-induced asthma. Clin Exp Allergy 1995;25:340-9.
51. Amano M, Ogawa H, Kojima K, Kamidaira T, Suetsugu S, Yoshihama M, Satoh T, Samejima T, Matsumoto I. Identification of the major allergens in wheat flour responsible for baker's asthma. Biochem J 1998;330:1229-34.
52. Fränken J, Stephan U, Meyer HE, König W. Identification of alpha-amylase inhibitor as a major allergen of wheat flour. Int Arch Allergy Immunol 1994;104:171-4.
53. Theobald K, Thiel H, Kallweit C, Ulmer W, König W. Detection of proteins in wheat flour extracts that bind human IgG, IgE, and mouse monoclonal antibodies. J Allergy Clin Immunol 1986;78:470-7.
54. Armentia A, Sanchez-Monge R, Gomez L, Barber D, Salcedo G. In vivo allergenic activities of eleven purified members of a major allergen family from wheat and barley flour. Clin Exp Allergy 1993;23:410-5.
55. García-Casado G, Armentia A, Sánchez-Monge R, Sánchez LM, Lopez-Otín C, Salcedo G. A major baker's asthma allergen from rye flour is considerably more active than its barley counterpart. FEBS Lett 1995;364:36-40.
56. García-Casado G, Armentia A, Sánchez-Monge R, Malpica JM, Salcedo G. Rye flour allergens associated with baker's asthma. Correlation between in vivo and in vitro activities and comparison with their wheat and barley homologues. Clin Exp Allergy 1996;26:428-35.
57. Barber D, Sánchez-Monge R, Gómez L, Carpizo J, Armentia A, López-Otín C, Juan F, Salcedo G. A barley flour inhibitor of insect alpha-amylase is a major allergen associated with baker's asthma disease. FEBS Lett 1989;248:119-22.
58. Sánchez-Monge R, García-Casado G, López-Otín C, Armentia A, Salcedo G. Wheat flour peroxidase is a prominent allergen associated with baker's asthma. Clin Exp Allergy 1997;27:1130-7.
59. Yamashita H, Nanba Y, Onishi M, Kimoto M, Hiemori M, Tsuji H. Identification of a wheat allergen, Tri a Bd 36K, as a peroxidase. Biosci Biotechnol Biochem 2002;66:2487-90.
60. Kobrehel K, Wong JH, Balogh A, Kiss F, Yee BC, Buchanan BB. Specific reduction of wheat storage proteins by thioredoxin h. Plant Physiol 1992;99:919-24.
61. Weichel M, Glaser AG, Ballmer-Weber BK, Schmid-Grendelmeier P, Crameri R. Wheat and maize thioredoxins: a novel cross-reactive cereal allergen family related to baker's asthma. J Allergy Clin Immunol 2006;117:676-81.
62. Salcedo G, Sánchez-Monge R, Barber D, Díaz-Perales A. Plant non-specific lipid transfer proteins: an interface between plant defence and human allergy. Biochim Biophys Acta 2007;1771:781-91.
63. Palacin A, Quirce S, Armentia A, Fernández-Nieto M, Pacios LF, Asensio T, Sastre J, Diaz-Perales A, Salcedo G. Wheat lipid transfer protein is a major allergen associated with baker's asthma. J Allergy Clin Immunol 2007;120:1132-8.
64. Palacin A, Varela J, Quirce S, del Pozo V, Tordesillas L, Barranco P, Fernandez-Nieto M, Sastre J, Diaz-Perales A, Salcedo G. Recombinant lipid transfer protein Tri a 14: a novel heat and proteolytic resistant tool for the diagnosis of baker's asthma. Clin Exp Allergy 2009;39:1267-76.
65. Enrique E, Ahrazem O, Bartra J, Latorre MD, Castelló JV, de Mateo

JA, Montoya E, Malek T, Barber D, Salcedo G. Lipid transfer protein is involved in rhinoconjunctivitis and asthma produced by rice inhalation. J Allergy Clin Immunol 2005;116:926-8.
66. Constantin C, Quirce S, Grote M, Touraev A, Swoboda I, Stoecklinger A, Mari A, Thalhamer J, Heberle-Bors E, Valenta R. Molecular and immunological characterization of a wheat serine proteinase inhibitor as a novel allergen in baker's asthma. J Immunol 2008; 180:7451-60.
67. Constantin C, Quirce S, Poorafshar M, Touraev A, Niggemann B, Mari A, Ebner C, Akerström H, Heberle-Bors E, Nystrand M, Valenta R. Micro-arrayed wheat seed and grass pollen allergens for component-resolved diagnosis. Allergy 2009;64:1030-7.
68. Lehto M, Airaksinen L, Puustinen A, Tillander S, Hannula S, Nyman T, Toskala E, Alenius H, Lauerma A. Thaumatin-like protein and baker's respiratory allergy. Ann Allergy Asthma Immunol 2010;104:139-46.
69. Liu JJ, Sturrock R, Ekramoddoullah AK. The superfamily of thaumatin-like proteins: its origin, evolution, and expression towards biological function. Plant Cell Rep 2010;29:419-36.
70. Walsh BJ, Howden ME. A method for the detection of IgE binding sequences of allergens based on a modification of epitope mapping. J Immunol Methods 1989;121:275-80.
71. Sandiford CP, Tatham AS, Fido R, Welch JA, Jones MG, Tee RD, Shewry PR, Newman Taylor AJ. Identification of the major water/salt insoluble wheat proteins involved in cereal hypersensitivity. Clin Exp Allergy 1997;27:1120-9.
72. Mittag D, Niggemann B, Sander I, Reese I, Fiedler EM, Worm M, Vieths S, Reese G. Immunoglobulin E-reactivity of wheat-allergic subjects (baker's asthma, food allergy, wheat-dependent, exercise-induced anaphylaxis) to wheat protein fractions with different solubility and digestibility. Mol Nutr Food Res 2004;48:380-9.
73. Snégaroff J, Branlard G, Bouchez-Mahiout I, Laudet B, Tylichova M, Chardot T, Pecquet C, Choudat D, Raison-Peyron N, Vigan M, Kerre S, Laurière M. Recombinant proteins and peptides as tools for studying IgE reactivity with low-molecular-weight glutenin subunits in some wheat allergies. J Agric Food Chem 2007;55: 9837-45.
74. Bittner C, Grassau B, Frenzel K, Baur X. Identification of wheat gliadins as an allergen family related to baker's asthma. J Allergy Clin Immunol 2008;121:744-9.
75. Baur X, Sauer W, Weiss W. Baking additives as new allergens in baker's asthma. Respiration 1988;54:70-2.
76. Baur X, Fruhmann G, Haug B, Rasche B, Reiher W, Weiss W. Role of Aspergillus amylase in baker's asthma. Lancet 1986;1:43.
77. Sander I, Raulf-Heimsoth M, Siethoff C, Lohaus C, Meyer HE, Baur X. Allergy to Aspergillus-derived enzymes in the baking industry: identification of beta-xylosidase from Aspergillus niger as a new allergen (Asp n 14). J Allergy Clin Immunol 1998;102:256-64.
78. Quirce S, Cuevas M, Díez-Gómez M, Fernández-Rivas M, Hinojosa M, González R, Losada E. Respiratory allergy to Aspergillus-derived enzymes in bakers' asthma. J Allergy Clin Immunol 1992;90: 970-8.
79. Quirce S, Fernández-Nieto M, Bartolomé B, Bombín C, Cuevas M, Sastre J. Glucoamylase: another fungal enzyme associated with baker's asthma. Ann Allergy Asthma Immunol 2002;89:197-202.
80. Merget R, Sander I, Raulf-Heimsoth M, Baur X. Baker's asthma due to xylanase and cellulase without sensitization to alpha-amylase and only weak sensitization to flour. Int Arch Allergy Immunol 2001;124:502-5.
81. Quirce S, Polo F, Figueredo E, González R, Sastre J. Occupational asthma caused by soybean flour in bakers--differences with soybean-induced epidemic asthma. Clin Exp Allergy 2000;30:839-46.
82. Quirce S, Fernández-Nieto M, Polo F, Sastre J. Soybean trypsin inhibitor is an occupational inhalant allergen. J Allergy Clin Immunol 2002;109:178.
83. Lavaud F, Perdu D, Prévost A, Vallerand H, Cossart C, Passemard F. Baker's asthma related to soybean lecithin exposure. Allergy 1994; 49:159-62.
84. Campbell CP, Yates DH. Lupin allergy: a hidden killer at home, a menace at work; occupational disease due to lupin allergy. Clin Exp Allergy 2010;40:1467-72.
85. Belchi-Hernandez J, Mora-Gonzalez A, Iniesta-Perez J. Baker's asthma caused by Saccharomyces cerevisiae in dry powder form. J Allergy Clin Immunol 1996;97:131-4.
86. Valdivieso R, Moneo I, Pola J, Muñoz T, Zapata C, Hinojosa M, Losada E. Occupational asthma and contact urticaria caused by buckwheat flour. Ann Allergy 1989;63:149-52.
87. Vandenplas O, Vander Borght T, Delwiche JP. Occupational asthma caused by sunflower-seed dust. Allergy 1998;53:907-8.
88. Escudero C, Quirce S, Fernández-Nieto M, Miguel J, Cuesta J, Sastre J. Egg white proteins as inhalant allergens associated with baker's asthma. Allergy 2003;58:616-20.
89. Revsbech P, Dueholm M. Storage mite allergy among bakers. Allergy 1990;45:204-8.
90. Armentia A, Tapias J, Barber D, Martin J, de la Fuente R, Sanchez P, Salcedo G, Carreira J. Sensitization to the storage mite Lepidoglyphus destructor in wheat flour respiratory allergy. Ann Allergy 1992;68:398-403.
91. Lunn JA, Hughes DT. Pulmonary hypersensitivity to the grain weevil. Br J Ind Med 1967;24:158-61.
92. Armentia A, Lombardero M, Martínez C, Barber D, Vega JM, Callejo A. Occupational asthma due to grain pests Eurygaster and Ephestia. J Asthma 2004;41:99-107.
93. Bobolea I, Barranco P, Pastor-Vargas C, Iraola V, Vivanco F, Quirce S. Arginine kinase from the cellar spider (Holocnemus pluchei): a new asthma-causing allergen. Int Arch Allergy Immunol 2011;155: 180-6.
94. Klaustermeyer WB, Bardana EJ Jr, Hale FC. Pulmonary hypersensitivity to Alternaria and Aspergillus in baker's asthma. Clin Allergy 1977;7:227-33.
95. Vandenplas O. Occupational asthma: etiologies and risk factors. Allergy Asthma Immunol Res 2011;3:157-67.
96. Armentia A, Martin-Santos JM, Quintero A, Fernandez A, Barber D, Alonso E, Gil I. Bakers' asthma: prevalence and evaluation of immunotherapy with a wheat flour extract. Ann Allergy 1990;65:265-72.
97. Swaminathan S, Heddle RJ. Wheat flour immunotherapy in baker's asthma. Intern Med J 2007;37:663-4.
98. Cirla AM, Lorenzini RA, Cirla PE. Specific immunotherapy and relocation in occupational allergic bakers. G Ital Med Lav Ergon 2007;29:443-5.
99. Olivieri M, Biscardo CA, Turri S, Perbellini L. Omalizumab in persistent severe bakers' asthma. Allergy 2008;63:790-1.
100. Pérez Pimiento A, Bueso Fernández A, García Loria J, Rodríguez Cabreros MI, Mosquera MR, García Cubero A. Effect of omalizumab treatment in a baker with occupational asthma. J Investig Allergol Clin Immunol 2008;18:490-1.

Aliment Pharmacol Ther 2001; 15: 1647–1653.

Immune sensitization to food, yeast and bacteria in Crohn's disease

J. VAN DEN BOGAERDE*, M. A. KAMM* & S. C. KNIGHT†
**St Mark's Hospital, London, UK; and †Immunopathology, Imperial College School of Medicine, London, UK*

Accepted for publication 13 March 2001

SUMMARY

Background: Complex food proteins and enteric flora may act as antigenic stimuli in Crohn's disease. This study assessed the prevalence and magnitude of lymphocyte priming to these antigens in Crohn's disease.
Methods: A total of 31 Crohn's disease patients (median age 42 years, range 25–72 years) and 22 healthy controls (median 29 years, 23–43 years) were studied. Peripheral blood lymphocytes were collected and incubated with antigens in hanging drop culture for 4 days. The antigens tested were cow's milk, cereals, cabbage group, citrus group, peanut group, *Saccharomyces* (yeast), *Bacteroides, E. coli* and *Klebsiella*. On the 4th day ^{3}H-thymidine incorporation was measured after a 4-h pulse. Responses to antigens were considered positive if mean proliferative values were above the 99% confidence interval for background proliferation.
Results: The mean background and mitogen-stimulated proliferation did not differ between patients and controls. The mean proliferation to antigens was not above background in controls, but in Crohn's patients proliferative responses to all food and bacterial antigens were significantly higher than background values. Twenty-three out of 31 Crohn's patients and five out of 22 controls ($P = 0.0003$) responded to one or more antigens. Sixteen Crohn's patients and two controls responded to four or more antigens ($P = 0.001$, Fisher's exact test).
Conclusion: The reactivity of peripheral lymphocytes to food, yeast and bacterial antigens, especially multiple antigens, is common in Crohn's disease. These sensitized lymphocytes may contribute to the inflammatory process.

INTRODUCTION

The aetiology of Crohn's disease has been extensively studied, but no consensus regarding the immunopathogenesis of this disease has been reached. It has been suggested that intraepithelial T-cells in patients with Crohn's disease are activated by an exogenous antigen, and damage to the mucosa is caused by an indirect 'bystander' mechanism.[1] Two possible sources for exogenous antigens in Crohn's disease are food and enteric flora.

Evidence in support of the importance of food antigens perpetuating the inflammatory process comes from studies showing that diets whose sole protein source consists of amino acids or simple proteins can induce remission in Crohn's disease.[2–9] Enteral feeding with these diets appears to be as effective as cortico-steroid therapy in treating active Crohn's disease.[7, 8] Clinically identifying food antigens which might initiate or exacerbate disease is extremely difficult, and requires utilizing diets which patients find difficult to tolerate. The induction of remission after elemental diet therapy, followed by reintroduction of food has been attempted in Crohn's disease, but in one study specific food sensitivity was confirmed in only three out of 42 patients.[10] We hypothesized that complex proteins in food prime immune cells, and that these cells can be identified using *in vitro* laboratory techniques.

Laboratory based experimental work has supported the hypothesis that food or bacterial antigens are involved

Correspondence to: Professor M. A. Kamm, St Mark's Hospital, Northwick Park, Watford Road, Harrow, Middlesex HA1 3UJ, UK.
E-mail: kamm@ic.ac.uk

in the pathogenesis of Crohn's disease. The proliferation of peripheral as well as lamina propria T lymphocytes to bacterial, mycobacterial and fungal antigens has previously been demonstrated.[11, 12] A serum assay measuring antibodies against anaerobic coccoid rods had a specificity of 89% for Crohn's disease but was not specific for ulcerative colitis.[13] Titres of mucosal IgG antibodies have been shown to be significantly higher in Crohn's disease and ulcerative colitis than in controls, and some of these antibodies have been shown to be directed against cytoplasmic antigens of commensal bacterial flora.[14] Mucosal lymphocytes from normal individuals are tolerant to autologous bacterial flora, but lymphocytes from patients with inflammatory bowel disease proliferate when co-cultured with autologous bacterial antigens.[12, 15] Serum antibodies and proliferative lymphocyte responses to Saccharomyces cerevisiae (bakers and brewers yeast) have been shown to be significantly increased in patients with Crohn's disease, when compared to patients with ulcerative colitis or healthy controls.[16, 27] We therefore hypothesized that patients with Crohn's disease may also be sensitized to enteric bacteria or yeasts.

Amongst the most compelling evidence implicating exogenous antigens in Crohn's disease is the demonstration of persistent $CD4^+$ T-cell clones in the peripheral blood of patients with Crohn's disease.[17] These T-cell clones are shared in patients with similar HLA types, suggesting a relationship between an exogenous antigen and a genetic background in producing T-cell clonal expansion leading to persistent mucosal damage.

The aim of the study was to test whether peripheral blood lymphocytes of patients with Crohn's disease respond to food or bacterial antigens in a way which differs from healthy controls. If *in vitro* laboratory techniques could be used to identify abnormal sensitivities to exogenous antigens, this may form the basis for instituting elimination diets or altering enteric flora as therapies in patients with Crohn's disease.

MATERIALS AND METHODS

Subjects

Proliferative responses were assessed in 22 healthy controls (median age 29 years, range 23–43 years) and 31 patients with Crohn's disease (median age 42 years, range 25–72 years). Of the 31 Crohn's disease patients, 25 had ileocolonic disease, four ileal disease, and two colonic disease. Ten had active disease, as measured by the Harvey–Bradshaw index, five were on no drug therapy, 19 used a 5-ASA preparation, 11 were on steroids, and seven used azathioprine.[18]

Approval was obtained from the Harrow Research Ethical Committee and all subjects gave informed consent.

Peripheral blood lymphocyte proliferation to food, yeast and bacterial antigens

Peripheral blood lymphocytes were prepared by collecting 15 mL of blood added to 0.5 mL (500 units) of Heparin. The blood was kept at room temperature until lymphocyte separation. Lymphocytes were isolated by centrifugation on a Ficoll gradient (Histopaque, Sigma). Lymphocytes were then aspirated from the interface and suspended in complete medium (RPMI-1640, Dutch modification, Sigma, UK) supplemented with 10% v/v heat inactivated foetal calf serum, 100 μg/mL L-glutamine, 100 U/mL penicillin, 100 μg/mL streptomycin and 5×10^{-5} M 2-mercaptoethanol. Triplicate 20 μL hanging drop cultures containing 50 000–100 000 peripheral blood cells per well were established in Terasaki plates and one antigen diluted 10-fold was added to each well.[19] Plates were inverted over saline and incubated for 4 days at 37 °C in a humidified atmosphere of 5% CO_2 in air. One microlitre (equivalent to 1 μg/mL of ^{3}H-thymidine at a specific activity of 2 Ci/mmol) was then added to each 20 μL well and incubated for a further 4 h. The cultures were then harvested by blotting on to filter papers. This technique, using a low specific-activity thymidine in flooding conditions for a short pulse time, results in low counts. These reflect the level of DNA synthesis without the complication of limiting the availability of thymidine or excessive radiation damage to cells.[19] Radioisotope incorporation was determined by liquid scintillation counting on a Beta counter.

Food antigens

Purified food antigens were purchased from Bayer (Spokane, Washington). The following groups of antigens were utilized:

1 Cereal grain mix (barley, corn, oat, rice, rye and wheat), 1/10 weight for volume.

2 Cabbage group mix (broccoli, brussel sprouts, cabbage and cauliflower), 1/10 weight for volume.
3 Citrus mix (grapefruit, lemon, lime and sweet orange), 1/10 weight for volume.
4 Whole cow's milk, 1/20 weight for volume.
5 Bakers yeast and brewers yeast (*Saccharomyces cerevisiae*), 1/10 weight for volume.
6 Peanut mix (runner peanut, Virginia, Spanish), 200 protein nitrogen units/mL.

Before use in lymphocyte culture assays, antigens were dialysed for 24 h in medium (RPMI-1640, Dutch modification, Sigma, UK), and then stored at 4 °C. Dialysis was undertaken in order to remove preservatives from the antigen solution, which would depress lymphocyte proliferation.

The following bacterial antigens were used: Bacteroides, Klebsiella and *E. coli*. The *E. coli* bacteria were derived from the NTCT strain 104, while Bacteroides and Klebsiella were derived from wild organisms. Bacteria were diluted to 10^8 organisms/mL in RPMI medium. Irradiation with 3000 Rad (Cesium 57 source) ensured that these organisms were not able to proliferate in culture. A 4-day incubation of bacteria in growth medium confirmed that proliferation of irradiated bacteria did not occur (as measured by ^{3}H-thymidine incorporation).

Statistical analysis

The 99% confidence interval for background proliferation was calculated. Antigen responses were considered to be positive if mean proliferation to a particular antigen, at a particular concentration, was above the upper limit of the 99% confidence interval for background proliferation. The combined 99% confidence interval and mean values were calculated for background, con A, and each antigen for patients and controls. The Fisher's exact test was used to compare the number of patients and controls responding to antigens.

RESULTS

Initial experiments were undertaken to determine the optimal incubation time, antigen concentrations and responder cell numbers. Optimal proliferation was recorded after 4 days in culture (data not shown). Proliferation to antigens varied with cell responder number, but good responses were seen when 50 000–100 000 responder cells were used per well. Some patients responded vigorously to certain antigens at 50 000 responder cells, and less vigorously at 100 000 responders. For the purposes of standardization, all experiments were undertaken with a constant responder cell number of 100 000 cells per well, and 10-fold dilution of antigens.

Individual data

The data for Crohn's disease patients are shown in Table 1 and for healthy controls in Table 2. Twenty-three out of 31 Crohn's disease patients responded to one or more of the antigens, while eight patients showed no response. The following positive proliferative responses were noted in Crohn's disease patients: 13 to milk, 16 to cabbage, 14 to cereal, nine to citrus, 16 to peanut, 18 to *E. coli*, 11 to Bacteroides, 10 to Klebsiella, six to bakers yeast, and nine to brewers yeast. Five out of 22 healthy controls responded to one or more antigens: one to milk, two to cabbage, two to peanut, one to *E. coli*, one to Klebsiella, one to bakers yeast, and three to brewers yeast.

The proportion of Crohn's disease patients responding to at least one antigen differed significantly from healthy controls (Fisher's exact test, $P = 0.0003$). Sixteen patients responded to four or more antigens, compared to two controls ($P = 0.001$, Fisher's exact test).

Pooled group data for Crohn's disease patients and controls

The proliferative responses for the pooled groups of patients and healthy controls were then assessed. The mean proliferative responses for these two groups are shown in Table 3.

The mean background proliferations (and 99% confidence intervals) of patients and healthy controls were 161 (252) and 246 (314), respectively. The mean proliferation for these two groups to con A was 828 and 868 counts, respectively. Neither background nor response to con A differed significantly between groups. In the healthy control group, none of the mean proliferative responses to antigens exceeded the upper 99% confidence interval of background. In the Crohn's disease patients, however, mean proliferation to all antigens exceeded the upper 99% confidence interval for background proliferation.

To determine whether those Crohn's disease patients with small bowel disease were more likely to respond to

Table 1. Individual data for Crohn's disease patients, $n = 31$, showing only the proliferative responses to antigens which were above the 99% confidence interval for the background for that patient. The upper 99% confidence interval value is shown in background column

Patient number	Upper 99% CI limit Background	Mean proliferative response to each antigen									
		Milk	Cabbage	Cereal	Citrus	Peanuts	*E. coli*	Bacteriodes	Klebsiella	Bake yeast	Brew yeast
C1	362		559				894	7639	8937		
C2	114						347		139		
C3	343	645	788			2649	491		2250		
C4	141	559	357			320	206				226
C5	57	1107	885	1179	317		386	212	324	384	242
C6	405										
C7	440		688			597					486
C8	128										
C9	1455						1473				
C10	58	503	912	309		677	250	192		120	330
C11	2010										
C12	105	440	20600	12690	26650	3846	7481				
C13	143		225	271		6181	299				
C14	2249		25410					4699	4677		
C15	82	1848	629	317	263	684	1081		441		
C16	112		469	346	206	432	170				158
C17	146			1880		339	752				
C18	146	456		1308	147	631		300	8482		
C19	268					294		6582	6260		
C20	376					674					454
C21	959	1249		1200	1753		1861	1590			2329
C22	111	446	401	348	468	1156	240	374	278	120	249
C23	199										
C24	48										
C25	241										
C26	328										
C27	340	638	655	575		1143	561	1396		486	768
C28	110										
C29	260	1240	1080	457		1440	458			360	444
C30	31	124	37	52	118		46	55			
C31	390	1869	1659	1840	806	2143	838	1497	2134	1529	1901

food or yeast, and those with large bowel disease were more likely to respond to colonic bacterial antigens, patients with small bowel or ileocolonic disease were compared to those with large bowel disease only. A positive response was taken as response to at least one food or yeast or bacterial antigen. Those with small bowel or ileocolonic disease responded to 37 bacterial and 81 food or yeast antigens. Those with colonic disease only ($n = 2$) responded to two bacterial and eight food or yeast antigens. Therefore, the distribution of disease did not appear to affect the relative frequency of responses to food, yeast or bacterial antigens. No statistical comparison was made.

The eight patients in the Crohn's disease group who did not respond to any antigen were also analysed separately. One of these non-responders (C28) was on high dose intravenous steroids, while another (C23) was on prednisolone 50 mg per day, and ciclosporin 50 mg per day. One non-responder with active disease (Harvey–Bradshaw score of 7) was on azathioprine 100 mg per day, and two patients with inactive disease were only using 5-amino salicylic acid (ASA) preparations. Three non-responders with inactive disease were on no medication.

The 23 patients from the Crohn's disease group who responded to one or more antigen were analysed separately. Response to antigen was not related to disease activity, or use of medications (Fisher's exact test for each of these parameters assessed separately, $P = 0.8$). It was not possible to test for each type of

Table 2. Individual data from healthy control subjects, $n = 22$, showing proliferative responses to antigens which were above the 99% confidence interval for the background. Mean responses above the 99% confidence interval were regarded as positive. The upper 99% confidence interval value is shown in background column

Subject number	Upper 99% CI limit Background	Mean proliferative response to each antigen									
		Milk	Cabbage	Cereal	Citrus	Peanut	*E. coli*	Bacteroides	Klebsiella	Bake yeast	Brew yeast
H1	1261										
H2	1383										
H3	708										
H4	1289										
H5	611	8917	800	1173		625					778
H6	669										
H7	238										
H8	1405										
H9	61									68	
H10	53										
H11	55										
H12	148		207	179			420		794		
H13	504										
H14	393										
H15	190										
H16	506										671
H17	1944										
H18	772										
H19	278										
H20	176										
H21	238										
H22	403										793

drug separately because of the small number of patients in each group.

DISCUSSION

This study has demonstrated a marked sensitization to a broad range of food, bacterial and yeast antigens in patients with Crohn's disease. An *in vitro* technique was used to identifying peripheral T-cells which proliferate after exposure to food, yeast and bacterial antigens. The hanging drop culture method used in these experiments has been shown to be a sensitive method of assaying secondary lymphocyte responses.[19] This technique produces substantial variability according to culture period, responder cell number, as well as the concentration of the stimulating antigen. For these reasons stringent criteria were used to define positive responses to antigen. Accordingly, mean responses to antigens were only considered positive if they were above the upper limit of the 99% confidence interval for background proliferation, as opposed to the usually applied 95% confidence interval.

The striking proliferation of lymphocytes derived from Crohn's disease patients was specific; the background proliferation and mitogen response did not differ between the groups studied. Occasional proliferative

Table 3. Group data for Crohn's disease patients ($n = 31$) and healthy controls ($n = 22$) after 4 days incubation with food, yeast and bacterial antigens. Mean proliferation is shown, with upper 99% confidence interval value in brackets next to mean. Positive proliferative responses for antigens above the upper 99% confidence interval for background proliferation are in bold

	Background	Con A	Milk	Cabbage	Cereal	Citrus	Peanut	*E. coli*	Bacteroides	Kleb	Yeast
Crohn's	161 (252)	**828**	**506**	**872**	**497**	**444**	**568**	**516**	**522**	**478**	**344**
Healthy controls	246 (314)	**868**	179	182	154	81	201	178	130	72	150

responses were observed in a minority of healthy controls; the importance of these isolated responses is not known. Presumably an intact mucosa prevents food antigens from coming into contact with stimulated T-cell clones in healthy controls.

Disease distribution, disease activity and the use of drug therapy, did not appear to influence the patients' lymphocyte responses to the antigens tested. The numbers were too small to be conclusive regarding the effect of high dose steroids or azathioprine.

Whether the lymphocyte sensitization to lumenal antigens in Crohn's disease is due to breached epithelium or is a part of the pathogenic process in some other way, such as a general immune cell hyper-responsiveness, is unknown. In a separate study we have tested the rectal mucosal blood flow responses to six food antigens in 10 Crohn's disease patients, using laser Doppler flowmetry.[20] Crohn's disease patients demonstrated abnormal rectal blood flow responses to yeast and citrus fruits, when group data were analysed. Individual Crohn's disease patients showed an abnormal response to all antigens, with rectal blood flow increasing in 24 out of 60 antigen tests, compared to six out of 60 tests in controls ($P < 0.0001$). These responses were specific to the gut, and were not seen with skin testing. These results suggest that the current *in vitro* studies are of direct clinical relevance. They also suggest a link between peripheral lymphocytes and events occurring in the gut, but not in the skin.

Previous studies addressing immune responsiveness to lumenal antigens in Crohn's disease have focused particularly on the prevalence and nature of antibodies to yeast. Anti Saccharomyces cerevisiae antibodies (ASCA) are strongly associated with Crohn's disease, but not ulcerative colitis.[21–23] In contrast, the prevalence of IgG anti-*Escherichia coli* antibodies is not significantly different between Crohn's disease and ulcerative colitis. The high prevalence of anti-yeast antibodies in Crohn's disease, and their low prevalence in ulcerative colitis, suggests that they are not raised solely due to antigenic exposure through a breached epithelium.

The antibody response to a number of food antigens has also been examined in a twin study of patients with inflammatory bowel disease. Twenty-six monozygotic twin pairs with inflammatory bowel disease and 52 healthy controls were investigated for serum antibodies (IgA, IgG, IgM) against ovalbumin, betalactoglobulin, gliadin, whole yeast (*Saccharomyces cerevisiae*) and yeast cell wall mannan.[24] Patients with ulcerative colitis were indistinguishable from healthy twins and controls, except for the response to gliadin. Both healthy and diseased twins had higher IgA levels to gliadin than controls. Twins who both had Crohn's disease displayed higher antibody titres of all antibody types (IgA, IgG and IgM) towards yeast cell wall mannan in particular, but also to whole yeast (*Saccharomyces cerevisiae*). In contrast, the response to gliadin, ovalbumin, and betalactoglobulin did not differ from healthy twins and was even lower than in the controls. The results argue against an increased systemic antigen presentation caused by an impaired mucosal barrier in inflammatory bowel disease. Rather, they suggest that yeast cell wall material (that is, mannan), or some antigen rich in mannose and cross-reacting with mannan, may play an aetiological role in Crohn's disease, but not in ulcerative colitis. The increases in IgA and IgM, as well as IgG, suggest that local and systemic immune systems are selectively activated by antigen(s) present in the cell wall of baker's yeast.

Food-specific IgE could not be detected in the sera from Crohn's disease patients, suggesting that alternative mechanisms are involved in the adverse response to food components.[25]

Previous studies have demonstrated an excessive proliferation of Crohn's patients' peripheral blood lymphocyte in response to yeast or milk.[26, 27]

If sensitized lymphocytes and specific antibodies play a pathogenic role, then exclusion of the relevant foods may provide therapeutic benefit, even in the short term. In a study of 19 patients with Crohn's disease, patients continued their usual diet for the first month (base-line period), but during the next 2 months dietary yeast was excluded. During one of these months, patients took baker's yeast capsules, while for the other month they took placebo capsules.[28] The mean Crohn's disease activity index (CDAI) whilst taking baker's yeast was significantly greater than during yeast exclusion. The mean maximum CDAI during yeast exclusion was significantly lower than during the base-line and baker's yeast inclusion periods. Patients with elevated yeast antibodies tended to develop a higher CDAI while receiving baker's yeast.

The clinical and long-term relevance of dietary antigen exposure has been examined in a recent study of patients with food intolerance who were re-exposed to previously eliminated foods more than 4 years after instituting an exclusion diet.[30] Provocation produced immune activation in peripheral blood lymphocytes, as

assessed by an increased release of interleukin-4 (IL-4), interferon gamma, and TNF-alpha.

In summary, peripheral lymphocytes of Crohn's disease patients are abnormally sensitized to food, yeast and bacterial antigens. These sensitized lymphocytes may contribute to the inflammatory process.

ACKNOWLEDGEMENTS

We are grateful to Dr C. Dore (Royal Post Graduate Medical School, Hammersmith Hospital, London) for statistical advice.

REFERENCES

1 Shanahan F. Current concepts of the pathogenesis of inflammatory bowel disease. Ir J Med Sci 1994; 163: 544–9.
2 Giaffer MH, North G, Holdsworth CD. Controlled trial of polymeric versus elemental diet in treatment of active Crohn's disease [see comments]. Lancet 1990; 335: 816–9.
3 Teahon K, Bjarnason I, Pearson M, Levi AJ. Ten years' experience with an elemental diet in the management of Crohn's disease. Gut 1990; 31: 1133–7.
4 O'Morain C, Segal AW, Levi AJ. Elemental diets in treatment of acute Crohn's disease. Br Med J 1980; 281: 1173–5.
5 Goode A, Hawkines T, Feggetter JG, Johnston ID. Use of an elemental diet for long-term nutritional support in Crohn's disease. Lancet 1976; 1: 122–4.
6 Jones VA. Comparison of total parenteral nutrition and elemental diet in induction of remission of Crohn's disease. Long-term maintenance of remission by personalized food exclusion diets. Dig Dis Sci 1987; 32: S100–7.
7 Sanderson IR, Udeen S, Davies PS, Savage MO, Walker Smith JA. Remission induced by an elemental diet in small bowel Crohn's disease. Arch Dis Child 1987; 62: 123–7.
8 O'Morain C, Segal AW, Levi AJ. Elemental diet as primary treatment of acute Crohn's disease: a controlled trial. Br Med J Clin Res Ed 1984; 288: 1859–62.
9 Mansfield JC, Giaffer MH, Holdsworth CD. Controlled trial of oligopeptide versus amino acid diet in treatment of active Crohn's disease. Gut 1995; 36: 60–6.
10 Pearson M, Teahon K, Levi AJ, Bjarnason I. Food intolerance and Crohn's disease [see comments]. Gut 1993; 34: 783–7.
11 D'Haens G, Hiele M, Rutgeerts P, Geboes K, Ceuppens JL. Depressed T cell reactivity to recall antigens in Crohn's disease before and after surgical resection. Gut 1994; 35: 1728–33.
12 Duchmann R, Marker Hermann E, Meyer zum Buschenfelde KH. Bacteria-specific T-cell clones are selective in their reactivity towards different enterobacteria or *H. pylori* and increased in inflammatory bowel disease. Scand J Immunol 1996; 44: 71–9.
13 Oudkerk Pool M, Bouma G, Meuwissen SG, *et al*. Serological markers to differentiate between ulcerative colitis and Crohn's disease. J Clin Pathol 1995; 48: 346–50.
14 Macpherson A, Khoo UY, Forgacs I, Philpott Howard J, Bjarnason I. Mucosal antibodies in inflammatory bowel disease are directed against intestinal bacteria. Gut 1996; 38: 365–75.
15 Duchmann R, Kaiser I, Hermann E, Mayet W, Ewe K, Meyer zum Buschenfelde KH. Tolerance exists towards resident intestinal flora but is broken in active inflammatory bowel disease (IBD) [see comments]. Clin Exp Immunol 1995; 102: 448–55.
16 Giaffer MH, Clark A, Holdsworth CD. Antibodies to *Saccharomyces cerevisiae* in patients with Crohn's disease and their possible pathogenic importance. Gut 1992; 33: 1071–5.
17 Probert CS, Chott A, Turner JR, *et al*. Persistent clonal expansions of peripheral blood CD4+ lymphocytes in chronic inflammatory bowel disease. J Immunol 1996; 157: 3183–91.
18 Harvey RF, Bradshaw JM. A simple index of Crohn's-disease activity. Lancet 1980; 1: 514.
19 Knight SC. Lymphocyte proliferation assays. In: Klaus GGB, ed. Lymphocytes: a Practical Approach. IRL Press Limited, Oxford, 1987: 189–207.
20 Bogaerde JB, Kamm MA, Cahill J, Emmanuel A, Vaizey C, Knight S. Immune response to food in Crohn's disease; *in vivo* and *in vitro* assessment. Gut 1998; 42(Suppl. 1): A86(Abstract).
21 Barnes RM, Allan S, Taylor-Robinson CH, Finn R, Johnson PM. Serum antibodies reactive with *Saccharomyces cerevisiae* in inflammatory bowel disease: is IgA antibody a marker for Crohn's disease? Int Arch Allergy Appl Immunol 1990; 92: 9–15.
22 Giaffer MH, Clark A, Holdsworth CD. Antibodies to *Saccharomyces cerevisiae* in patients with Crohn's disease and their possible pathogenic importance. Gut 1992; 33: 1071–5.
23 Quinton JF, Sendid B, Reumaux D, *et al*. Anti-*Saccharomyces cerevisiae* mannan antibodies combined with antineutrophil cytoplasmic autoantibodies in inflammatory bowel disease: prevalence and diagnostic role disease. Gut 1998; 42: 788–91.
24 Lindberg E, Magnusson KE, Tysk C, Jarnerot G. Antibody (IgG, IgA, and IgM) to baker's yeast (*Saccharomyces cerevisiae*), yeast mannan, gliadin, ovalbumin and betalactoglobulin in monozygotic twins with inflammatory bowel disease. Gut 1992; 33: 909–13.
25 Huber A, Genser D, Spitzauer S, Scheiner O, Jensen-Jarolim E. IgE/anti-IgE immune complexes in sera from patients with Crohn's disease do not contain food-specific IgE. Int Arch Allergy Immunol 1998; 115: 67–72.
26 Frieri M, Claus M, Boris M, Zitt M, Scalise D, Harris N. Preliminary investigation on humoral and cellular immune responses to selected food proteins in patients with Crohn's disease. Ann Allergy 1990; 64: 345–51.
27 Young CA, Sonnenberg A, Burns EA. Lymphocyte proliferation response to baker's yeast in Crohn's disease. Digestion 1994; 55: 40–3.
28 Barclay GR, McKenzie H, Pennington J, Parratt D, Pennington CR. The effect of dietary yeast on the activity of stable chronic Crohn's disease. Scand J Gastroenterol 1992; 27: 196–200.
29 Jacobsen MB, Aukrust P, Kittang E, *et al*. Relation between food provocation and systemic immune activation in patients with food intolerance. Lancet 2000; 356: 400–1.

JOURNAL OF CLINICAL MICROBIOLOGY, June 2004, p. 2840–2842 Vol. 42, No. 6
0095-1137/04/$08.00+0 DOI: 10.1128/JCM.42.6.2840–2842.2004

Use of Paraffin-Embedded Tissue for Identification of *Saccharomyces cerevisiae* in a Baker's Lung Nodule by Fungal PCR and Nucleotide Sequencing

Ping Ren,[1] Sundara Sridhar,[2] and Vishnu Chaturvedi[1,3]*

Mycology Laboratory, Wadsworth Center, New York State Department of Health,[1] and Department of Biomedical Sciences,[3] SUNY School of Public Health, University at Albany, Albany, and Department of Pathology, Coney Island Hospital, Brooklyn,[2] New York

Received 17 November 2003/Returned for modification 16 December 2003/Accepted 4 March 2004

A 40-year-old healthy male employed in a bakery presented with a single lung nodule and underwent investigations to rule out pulmonary carcinoma. Biopsy was positive for yeast cells, which did not match common fungal pathogens. PCR assay of paraffin-embedded tissue and nucleotide sequencing with ribosomal *ITS1-ITS2* universal primers revealed the presence of *Saccharomyces cerevisiae*.

Identification of fungal pathogens in histological sections frequently requires application of specialized stains (6). Many pathogenic yeasts appear as budding, rounded cells without any characteristic tissue forms (9). This situation is alleviated in instances in which the incriminating fungus can be isolated in culture. However, tissue specimens are not always available for culture. Recently, the application of PCR and nucleotide sequencing has been extended for identification of pathogenic fungi in histological sections. The paraffin-embedded tissue is used as a source of template DNA for a PCR assay with universal fungal ribosomal gene primers and/or a nested PCR assay with pathogen-specific primers, and the amplicons are then analyzed by restriction fragment length polymorphism and/or nucleotide sequencing for confirmation of fungal identity (2–5, 8, 11, 13). This approach is very promising in diagnostics, as it could lead to conclusive identification of the causal pathogen independently of histological or culture observations. We describe a case of a lung nodule in a healthy male that proved to be histologically negative for suspected lung carcinoma and instead revealed budding yeast cells, which were confirmed as *Saccharomyces cerevisiae* by PCR and nucleotide sequencing.

A 40-year-old healthy male was referred to the surgeon at Coney Island Hospital for a lung nodule discovered during a routine chest X-ray done as part of an annual physical examination. The patient was a nonsmoker with no history of any medical illness. A wedge resection of the lung was performed. A 0.7-cm-diameter solid grey-tan nodule was present in the lung parenchyma. The edges of the lesion were sharply demarcated from the surrounding normal lung parenchyma without any calcification. Histopathologic examination revealed an inflammatory mass composed of a background of fibrotic tissue with a moderately dense population of inflammatory cells composed of an equal admixture of histiocytes and lymphocytes. No discrete areas of necrosis were noted. Numerous oval-to-spherical structures reminiscent of fungal cells were revealed by hematoxylin-and-eosin staining (Fig. 1A). A silver methenamine stain confirmed that these were fungal cells, variable in size from 5 to 15 μm, mostly extracellular with occasional budding (Fig. 1B). Since the tissue was not saved for fungal culture, identification of the cells was first attempted on the basis of their morphology. The usual pathogenic fungi causing pulmonary nodular lesions are *Histoplasma capsulatum*, *Blastomyces dermatitidis*, *Coccidioides immitis*, and *Cryptococcus neoformans*. The larger size and predominant extracellular location were atypical for *H. capsulatum*, the absence of broad-based budding ruled out *B. dermatitidis*, and the absence of a mucinous capsule ruled out *C. neoformans*. Also, the size and morphology were inconsistent with *Coccidioides* species. *Paracoccidiodes brasiliensis* was also a consideration because of travel to South America; however, the morphology was not supportive. The presence of budding cells ruled out the possibility of *Pneumocystis carinii*. No other microorganisms were identified in the lesion.

The specimen was submitted to the Mycology Laboratory at the Wadsworth Center for further investigations. The lung tissue block was sliced into thin pieces with a sterile surgical blade and transferred to a microcentrifuge tube. A 200-μl aliquot of xylene (Sigma, St. Louis, Mo.) was added, mixed by inversion, heated for 15 min at 37°C, and centrifuged at 14,000 rpm in an Eppendorf model 5415 D centrifuge for 15 min. The supernatant was removed, a fresh 200-μl aliquot of xylene was added, and the whole step was repeated once. The pellet was washed twice with 1.0 ml of 100% ethanol for 30 min at 37°C to remove residual xylene. The ethanol was removed by centrifugation for 15 min, and the tissue pellet was air dried for DNA extraction. The QIAamp DNA Mini Kit (Qiagen, Valencia, Calif.) was used to extract the DNA from the tissue pellet in accordance with the manufacturer's protocol. PCR was performed with universal primers designed to amplify the *ITS1* region of the fungal ribosomal DNA. The oligonucleotide sequences were 5′-TCCGTAGGTGAACCTGCGG-3′ and 5′-GCTGCGTTCTTCATCGATGC-3′ (12). The thermal cycling conditions were initial denaturation at 95°C for 5 min, followed

* Corresponding author. Mailing address: Mycology Laboratory, Wadsworth Center, New York State Department of Health, SUNY School of Public Health, University at Albany, Albany, NY 12201-2002. Phone: (518) 453-6412. Fax: (518) 486-7811. E-mail: vishnu@wadsworth.org.

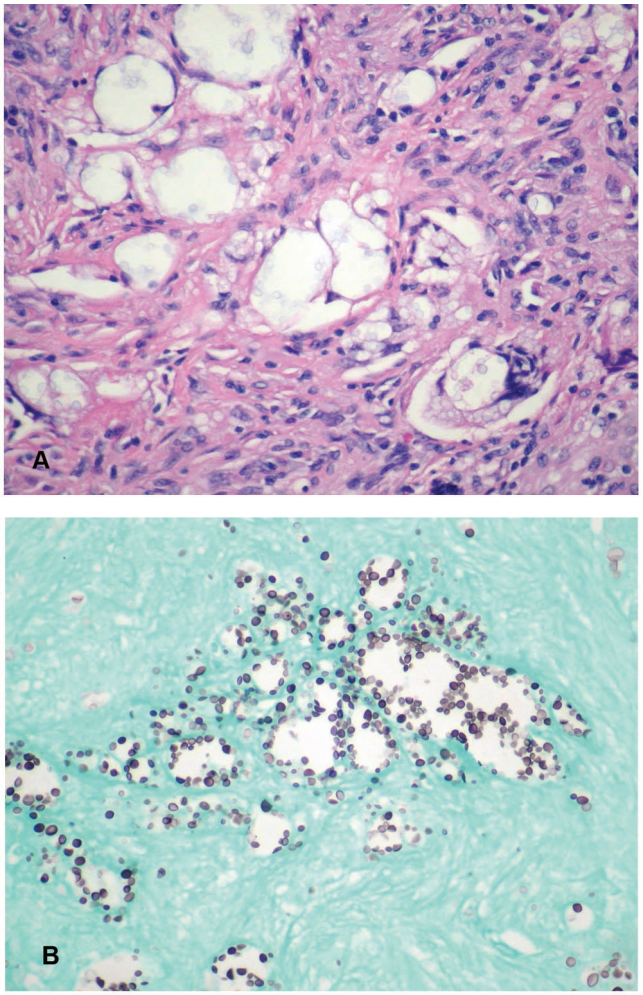


FIG. 1. Histological examination of lung nodule. (A) Hematoxylin-and-eosin staining revealing budding round-to-oval structures in the midst of inflammatory cells (lymphocytes and plasma cells). Original magnification, ×400. (B) Silver methenamine staining revealing extracellular fungal cells with occasional budding. Original magnification, ×400.

by 35 cycles of denaturation at 94°C for 1 min, annealing at 62°C for 30 s, and extension at 72°C for 1 min and a final extension step of 72°C for 10 min. The same reaction conditions were subsequently used to amplify the *ITS1-ITS2* regions with the primers 5′-TCCGTAGGTGAACCTGCGG-3′ and 5′-TCCTCCGCTTATTGATATGC-3′ (12). The DNA template was also used for PCR amplification of the human androgen receptor gene as a control with primers AR-F (5′-GC CTGTTGAACTCTTCTGAGC-3′) and AR-R (5′-GCTGTG AAGGTTGCTGTTCCTC-3′) (10). The control human DNA sample was prepared from blood. The PCR products were electrophoresed on 1.2% agarose gel in Tris-borate-EDTA buffer. The DNA amplicons were purified from the gel with a QIAquick gel extraction kit (Qiagen) and sequenced with an ABI PRISM 377 sequencer and a BigDye terminator cycle sequencing ready reaction kit (Applied Biosystems, Foster City, Calif.).

The template DNA was successfully extracted from the paraffin-embedded lung tissue, and PCRs worked well with both fungal and human primers (Fig. 2). One prominent DNA band of approximately 400 bp was obtained with *ITS1* universal fungal primers and not with the control human DNA (lanes 2 and 3), which indicated that only the lung nodule sample had fungal DNA. The positive bands of approximately 475 bp in lanes 4 and 5 were obtained with human androgen receptor primers from both lung nodule DNA and control human DNA, which indicated that the extracted DNA was comparable in quality to control human DNA prepared in the laboratory. Nucleotide sequencing of the DNA amplicon from lane 2 and homology searches in the GenBank database revealed the highest homology with the corresponding *S. cerevisiae* sequences. The subsequent sequencing of the 840-bp *ITS1-ITS2* fragment from lung nodule DNA also revealed the highest homology with *S. cerevisiae*.

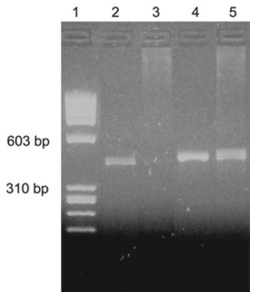


FIG. 2. Electrophoresis of DNA amplified by PCR of lung nodule. Lanes: 1, φX174 RF/HaeII DNA fragment used as a size standard; 2, positive PCR result with lung nodule DNA amplified by fungal *ITS1* primers; 3, negative PCR result with control human DNA and fungal *ITS1* primers; 4 and 5, positive PCR results with DNA from lung nodule and control human DNA amplified with primers for the human androgen receptor.

It is reasonable to suggest that the lung nodule in this patient was caused by inhalation of dry baking yeast powder since the patient must have been exposed during his work, which involved setting up bakeries in different areas. The patient did not receive any further treatment after surgery, and additional blood tests did not reveal any immune dysfunction. The patient is doing well and has been disease free for 2 years. Although *S. cerevisiae* is a rare fungal pathogen, mostly in immunocompromised patients, sporadic cases of vaginitis and asthma have been attributed to occupational exposure (1, 7, 14).

A number of investigators have described the use of a nested-PCR approach combining *ITS* universal primers with species-specific primers to confirm the etiology of *B. dermatitidis* and *H. capsulatum* in paraffin-embedded tissue (2, 3, 8, 11). This approach increases the specificity of the PCR and obviates the need for subsequent sequencing to confirm identity, but it cannot be used with samples containing unknown fungi. Two other groups have reported detailed comparisons of PCR with *ITS* primers, followed by hybridization with species-specific nucleic acid probes and/or nucleotide sequencing (5, 13). In one of these studies, it was found that hybridization with specific probes was rather limited in its sensitivity and specificity compared to nucleotide sequencing (13). In conclusion, the present report highlights the pathogenic potential of *S. cerevisiae* in healthy humans who may acquire the fungus by

occupational exposure. Our laboratory findings also confirm that the combination of PCR and nucleotide sequencing with *ITS1-ITS2* primers is a viable option for the identification of fungal pathogens from paraffin-embedded tissue, especially when the identity of the fungus is unknown.

Nucleotide sequence accession numbers. The sequences determined in this study have been deposited in the GenBank database with accession numbers AF500488 and AY525600.

We thank Ann Messer (Molecular Genetics Program, Wadsworth Center) for providing the DNA sample and primers for the human androgen receptor. The nucleotide sequencing was done at the Molecular Genetics Core of the Wadsworth Center.

REFERENCES

1. **Belchi-Hernandez, J., A. Mora-Gonzalez, and J. Iniesta-Perez.** 1996. Baker's asthma caused by *Saccharomyces cerevisiae* in dry powder form. J. Allergy Clin. Immunol. **97:**131–134.
2. **Bialek, R., A. C. Cirera, T. Herrmann, C. Aepinus, V. I. Shearn-Bochsler, and A. M. Legendre.** 2003. Nested PCR assays for detection of *Blastomyces dermatitidis* DNA in paraffin-embedded canine tissue. J. Clin. Microbiol. **41:**205–208.
3. **Bialek, R., A. Feucht, C. Aepinus, G. Just-Nubling, V. J. Robertson, J. Knobloch, and R. Hohle.** 2002. Evaluation of two nested PCR assays for detection of *Histoplasma capsulatum* DNA in human tissue. J. Clin. Microbiol. **40:**1644–1647.
4. **Hagari, Y., S. Ishioka, F. Ohyama, and M. Mihara.** 2002. Cutaneous infection showing sporotrichoid spread caused by *Pseudallescheria boydii* (*Scedosporium apiospermum*): successful detection of fungal DNA in formalin-fixed, paraffin-embedded sections by seminested PCR. Arch. Dermatol. **138:**271–272.
5. **Hendolin, P. H., L. Paulin, P. Koukila-Kahkola, V. J. Anttila, H. Malmberg, M. Richardson, and J. Ylikoski.** 2000. Panfungal PCR and multiplex liquid hybridization for detection of fungi in tissue specimens. J. Clin. Microbiol. **38:**4186–4192.
6. **Kwon-Chung, K. J., and J. A. Bennett.** 1992. Medical mycology. Lea & Febiger, Philadelphia, Pa.
7. **Nyirjesy, P., J. A. Vazquez, D. D. Ufberg, J. D. Sobel, D. A. Boikov, and H. R. Buckley.** 1995. Saccharomyces cerevisiae vaginitis: transmission from yeast used in baking. Obstet. Gynecol. **86:**326–329.
8. **Rivasi, F., B. Casali, A. Nanetti, G. Collina, and A. Mazzoni.** 2001. *Histoplasma capsulatum* var. *capsulatum* occurring in an HIV-positive Ghanaian immigrant to Italy: identification of *H. capsulatum* DNA by PCR from paraffin sample. APMIS **109:**721–725.
9. **Salfelder, K., T. R. de Liscano, and E. Sauerteig.** 1990. Atlas of fungal pathology, vol. 17. Kluwer Academic Publishers, Dordrecht, The Netherlands.
10. **Tilley, W. D., M. Marcelli, and M. J. McPhaul.** 1990. Expression of the human androgen receptor gene utilizes a common promoter in diverse human tissues and cell lines. J. Biol. Chem. **265:**13776–13781.
11. **Ueda, Y., A. Sano, M. Tamura, T. Inomata, K. Kamei, K. Yokoyama, F. Kishi, J. Ito, Y. Mikami, M. Miyaji, and K. Nishimura.** 2003. Diagnosis of histoplasmosis by detection of the internal transcribed spacer region of fungal rRNA gene from a paraffin-embedded skin sample from a dog in Japan. Vet. Microbiol. **94:**219–224.
12. **White, T. J., T. Bruns, S. Lee, and J. Taylor.** 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Academic Press, New York, N.Y.
13. **Willinger, B., A. Obradovic, B. Selitsch, J. Beck-Mannagetta, W. Buzina, H. Braun, P. Apfalter, A. M. Hirschl, A. Makristathis, and M. Rotter.** 2003. Detection and identification of fungi from fungus balls of the maxillary sinus by molecular techniques. J. Clin. Microbiol. **41:**581–585.
14. **Wilson, J. D., B. M. Jones, and G. R. Kinghorn.** 1988. Bread-making as a source of vaginal infection with *Saccharomyces cerevisiae*: report of a case in a woman and apparent transmission to her partner. Sex. Transm. Dis. **15:** 35–36.

Allergy 1998: 53: 506–512
Printed in UK – all rights reserved

ALLERGY
ISSN 0105-4538

IgE, IgA, and IgG responses to common yeasts in atopic patients

Savolainen J, Kortekangas-Savolainen O, Nermes M, Viander M, Koivikko A, Kalimo K, Terho EO. IgE, IgA, and IgG responses to common yeasts in atopic patients.
Allergy 1998: 53: 506–512. © Munksgaard 1998.

This study was undertaken to analyze the differences in exposure and sensitization to five common environmental yeasts. The responses of IgG, IgA, and IgE to *Candida albicans*, *C. utilis*, *Cryptococcus albidus*, *Rhodotorula rubra*, and *Saccharomyces cerevisiae* and purified *S. cerevisiae* enolase were analyzed by immunoblotting (IgE-IB), and the cross-reactivity of their IgE-binding components by IgE-IB inhibition. Twenty atopic subjects, with asthma, allergic rhinitis, or atopic dermatitis were included. In skin prick tests (SPT), 12 of the patients showed simultaneous reactivity to at least two of the five yeasts, four reacted to one of the yeasts, and four had no responses. Antigens run in SDS–PAGE and transferred to nitrocellulose were probed with enzyme-labeled IgA-, IgG-, and IgE-specific antibodies. The IgE immunoblotting revealed most IgE-binding bands in *C. albicans* (11 bands) followed by *C. utilis* (eight bands), *S. cerevisiae* (five bands), *R. rubra* (five bands), and *Cr. albidus* (four bands). Six of the IgE-binding bands of *C. albicans* and *C. utilis* shared molecular weight, and only two bands shared molecular weight with other yeasts. These were the 46-kDa band, shared by all five yeasts, and a 13-kDa band shared by four yeasts. Prominent IgE binding was seen to a 46-kDa band of *C. albicans* (seven patients), *C. utilis* (five patients), and *S. cerevisiae* (one patient) and to corresponding weak bands of *Cr. albidus* and *R. rubra* (one patient). The possible cross-reactivity of the 46-kDa band was analyzed by IgE-IB inhibition and densitometry, revealing clear *C. albicans* inhibition of *C. utilis* (80%) and enolase (98%) (autoinhibition 100%). The strongest IgG responses were seen against *S. cerevisiae* and *C. albicans*. The responses were mainly against mannans of *C. albicans* and *S. cerevisiae*, suggesting that most of the exposure is to these yeasts. Yeasts with different types of exposure, from saprophytic growth on human mucous membranes to exposure by air and food, were shown to cross-react at the allergenic level. Atopic patients primarily sensitized by *C. albicans* and *S. cerevisiae* may develop allergic symptoms by exposure to other environmental yeasts due to cross-reacting IgE antibodies.

J. Savolainen[1,2,5], O. Kortekangas-Savolainen[3], M. Nermes[2], M. Viander[4], A. Koivikko[2], K. Kalimo[3], E. O. Terho[1]

Departments of [1]Pulmonary Diseases and Clinical Allergology, [2]Pediatrics, [3]Dermatology, and [4]Medical Microbiology, Turku Allergy Research Unit, MediCity Research Laboratory, University of Turku, Turku, Finland; [5]INSERM U 454, Hôpital Arnaud de Villeneuve, Montpellier France

Key words: allergen; *Candida albicans*; *Candida utilis*; cross-reactivity; *Cryptococcus albidus*; enolase; *Rhodotorula rubra*; *Saccharomyces cerevisiae*.

Johannes Savolainen
Medicity, Turku University
Tykistökatu 6
FIN-20520 Turku
Finland

Accepted for publication 22 December 1997

Several yeasts have been found to play a role in allergic diseases. *Candida albicans*, a common saprophyte of the mucous membranes, has been associated with bronchial asthma, allergic rhinitis, chronic urticaria and atopic dermatitis (1–5). Atopic allergy to a lipophilic yeast, *Pityrosporum ovale*, growing on the skin has been connected with exacerbations of atopic dermatitis (6, 7). *Saccharomyces cerevisiae*, the familiar baker's yeast, is widely used in foods. *S. cerevisiae* may also be important in the pathogenesis of atopic dermatitis as well as chronic urticaria (3, 8).

Despite evident antigenic cross-reactivity, the cross-reacting allergens of yeasts have remained unexamined until recently (9, 10). The allergenic potencies of mannan and several cytoplasmic proteins of *C. albicans*, *P. ovale*, and *S. cerevisiae* have been confirmed (11–18). Immunoblotting and RAST-inhibition studies have revealed cross-reacting allergens in the mannan and protein

allergens of these yeasts. A 46–48-kDa enolase is a main allergen and cross-reacting component of *C. albicans* and *S. cerevisiae* (14, 19–22). Mannan polysaccharide is a cross-reacting allergen of *C. albicans*, *P. ovale*, and *S. cerevisiae* (23–25).

Candida utilis and *Candida tropicalis*, of industrial origin, have been associated with allergic symptoms (26). *Cryptococcus albidus* is the most common airborne yeast in Finland, followed by *Rhodotorula* species (27). There are no previous data on the allergens of *Rhodotorula*, *Cryptococcus*, or *C. utilis*.

This study was undertaken to analyze the differences in exposure and sensitization to five common yeasts by IgE, IgA, and IgG immunoblotting, as well as their allergenic cross-reactivity by immunoblotting inhibition.

Material and methods

Patients

Sixteen atopic patients with varying skin prick test (SPT) responses to the five studied yeasts (Table 1) were included in the study. Four atopic patients not responding to yeasts served as controls. The SPT were performed according to Nordic recommendations, as previously described (28). The serum samples were taken at the time of SPT and stored at –20°C until used. All patients had diagnoses of asthma, allergic rhinitis, or atopic dermatitis mainly in combinations (Table 1). Pooled sera from four nonatopic subjects were used as control in immunoblotting.

Yeast extracts

Four different yeast strains were cultured for these studies, as previously described (28). *C. albicans* strain 72024, *R. rubra* strain 70765, and *Cr. albidus* strain 71078 were supplied and typed by the Mycological Laboratory of the Department of Dermatology, University of Turku (Finland). *C. utilis*, strain VTT-C-71015 was obtained from the Department of Clinical Microbiology, University of Kuopio (Finland). *S. cerevisiae* cultures were provided by OY Alko AB, Helsinki, Finland (courtesy of Matti Korhola, Microbiological Laboratory of Alko). The extracts used for immunoblotting were prepared from lyophilized yeast cells, as previously described (28). Commercially available purified *S. cerevisiae* enolase was purchased from Sigma Chemicals (Sigma E 6126). Chemically purified *C. albicans* mannan was prepared according to the Peat method, as previously described (24).

SDS–PAGE and immunoblotting

Antigens and molecular weight markers (Electrophoresis Low Molecular Weight Calibration Kit, Pharmacia Fine Chemicals, Uppsala, Sweden) were separated with SDS–PAGE in 5–17.5% gradient gel according to Laemmli (29). Separated antigens were transferred to nitrocellulose sheets (Millipore 0.22 µm, Millipore, Bedford, MA, USA) in Transphor apparatus (LKB, Bromma, Sweden) according to Towbin et al. (30). IgA- and IgG-class antibody detection was performed with horseradish peroxidase (HRP)-conjugated anti-IgA and anti-IgG

Table 1. SPT, IgE-immunoblotting, and mannan-RAST results of study patients

Patient no.	Sex	Age (years)	Diagnosis[a]	*C. albicans*			*C. utilis*		*R. rubra*		*S. cerevisiae*		*Cr. albidus*	
				Man[b]	SPT[c]	IgE[d]	SPT	IgE	SPT	IgE	SPT	IgE	SPT	IgE
1	F	6	A, R, E	**0.65**	+	+	+	+	+	+	+	+	+	+
2	M	9	A, E	0.22	+	+	+	+	+	+	+	+	+	+
3	F	16	R, E	**0.60**	+	+	+	+	+	+	+	+	+	–
4	F	18	E	**1.27**	+	+	+	+	+	+	+	–	+	–
5	M	33	E	**0.64**	+	+	+	+	+	–	+	–	+	–
6	F	17	A, R, E	**9.52**	+	+	+	+	+	–	+	–	+	+
7	M	26	R, E	**0.41**	+	+	+	–	+	+	+	+	+	–
8	F	20	E	n.d.	+	–	+	–	+	–	+	–	+	–
9	F	15	A, R, E	**4.40**	+	+	+	+	+	+	+	–	–	–
10	M	37	E	**0.94**	+	+	–	–	+	–	+	–	–	–
11	M	14	R, E	0.16	+	+	–	–	+	+	–	–	–	–
12	F	17	R	**0.61**	+	+	–	+	–	–	–	–	–	–
13	F	17	E	**0.62**	+	+	–	–	–	–	–	–	–	–
14	F	45	E	0.32	+	+	–	–	–	–	–	–	–	–
15	F	16	R	n.d.	–	+	+	+	+	+	+	–	+	+
16	F	15	A, E	n.d.	–	–	–	–	+	–	–	–	–	–
17	F	35	R	n.d.	–	–	–	–	–	–	–	–	–	–
18	F	16	R	0.18	–	–	–	–	–	–	–	–	–	–
19	M	11	R	n.d.	–	–	–	–	–	–	–	–	–	–
20	M	16	A, E	n.d.	–	–	–	–	–	–	–	–	–	–
Total SPT/IgE[e]					14	14	10	9	13	8	11	4	9	4

[a]Diagnosis: A=asthma; R=rhinitis; E=eczema.
[b]Mannan-RAST index (positive values in bold).
[c]Skin prick test result.
[d]IgE response in immunoblotting.
[e]Total number of patients positive in SPT/IgE.

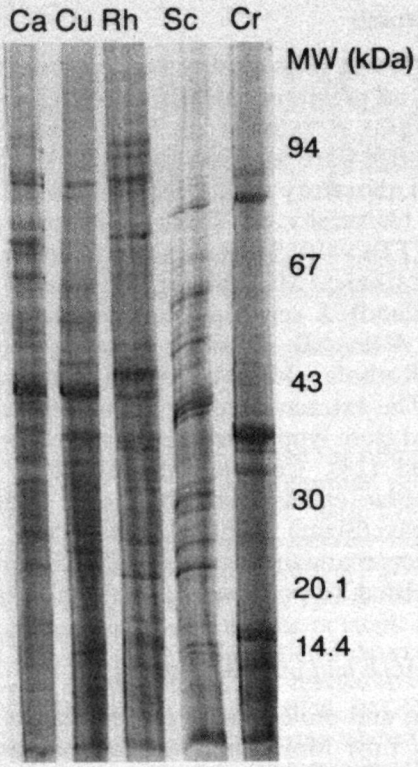


Fig. 1. Extracts of five study yeasts, *Candida albicans* (Ca), *C. utilis* (Cu), *Rhodotorula rubra* (Rh), *Saccharomyces cerevisiae* (Sc), and *Cryptococcus albidus* (Cr), run in 5–17.5% gradient SDS–PAGE gel and stained with Coomassie brilliant blue. Molecular weight markers (MW) are shown on right.

antibodies (DAKO Immunoglobulins, Denmark), as previously described (18). The IgE detection was done according to Bengtson et al. with β-galactosidase-labeled anti-IgE antibodies (Phadezym-RAST, Pharmacia Diagnostics, Uppsala, Sweden), as previously described (31). In IgE-immunoblotting inhibition prior to the incubation with the nitrocellulose strips, the sera were incubated with serial dilutions of the antigens for 30 min at 37°C.

Nitrocellulose RAST

The IgE antibodies against *C. albicans* mannan were analyzed with nitrocellulose RAST, as previously described (24).

Results

The protein profiles of the study extracts of all five yeasts run in a 5–17.5% gradient SDS–PAGE gel stained with Coomassie brilliant blue are presented in Fig. 1. The greatest similarity in the protein profiles was seen between *C. albicans* and *C. utilis*, which both showed a dominant protein staining of 46 kDa. In IgE immunoblotting, prominent IgE binding to the 46-kDa band was seen in one serum (patient 1) not only to *C. albicans* and *C. utilis*, but also to *S. cerevisiae*, as well as to, a lesser extent, to corresponding weak bands of *Cr. albidus* and *R. rubra* (Fig. 2).

Fig. 2 presents the parallel IgE immunoblots of all the five yeasts of five patients, who showed simultaneous strong IgE binding to at least three of the five yeasts. A comparison of the IgE immunoblotting, mannan-RAST, and SPT results of the patients presented, together with the clinical diagnosis, age, and sex, is shown in Table 1, and the summary of all the IgE-binding bands of all the five yeasts in Table 2. The yeast most often positive in IgE immunoblotting was *C. albicans* with 14 positive sera, followed by *C. utilis* (nine sera) and *R. rubra* (eight sera). Among SPT-positive patients, IgE antibodies were most often found against these yeasts, whereas IgE antibodies against both *S. cerevisiae* and *Cr. albidus* were seen in only four sera, in clearly less than 50% of the SPT-positive patients.

The IgE immunoblotting also revealed most IgE-binding bands in *C. albicans* (11 bands), again followed by *C. utilis* (eight bands). Five IgE-binding bands were seen in *R. rubra* and *S. cerevisiae* and four in *Cr. albidus*. Six IgE-binding bands of *C. albicans* and *C. utilis* had the same molecular weight. In general, only two bands shared molecular weight with other yeasts. These bands were the 46-kDa band, shared by all five yeasts, and a 13-kDa band shared by four yeasts.

Since the 46-kDa allergen was a major allergen of both *C. albicans* and *C. utilis* in this population, the possible cross-reactivity of it was analyzed by immunoblotting inhibition. Fig. 3 shows the inhibition of the 46-kDa bands of *C. albicans* and *C. utilis* and purified commercial *S. cerevisiae* enolase with individual serum from patient 1, who had an IgG response to *C. utilis*. For elimination of the effect of background differences, the net integrated intensities of the bands were assessed densitometrically and inhibition percentages were calculated. The *C. albicans* inhibition of *C. utilis* clearly reached 80% inhibition, the corresponding concentration giving a full 100% autoinhibition of *C. albicans*. The enolase staining was almost as completely inhibited (98%), with *C. albicans* as the autoinhibition. *S. cerevisiae* enolase inhibited *C. albicans* staining to 55% and *C. utilis* staining to only 15%, while the same concentration gave an enolase autoinhibition of 98%.

Examples of the IgG immunoblotting with the five yeasts are presented in Fig. 4, and the frequency of IgA and IgG antibodies against the yeasts in total and against mannan is shown in Table 3. The strongest IgG responses were seen against *S. cerevisiae* and

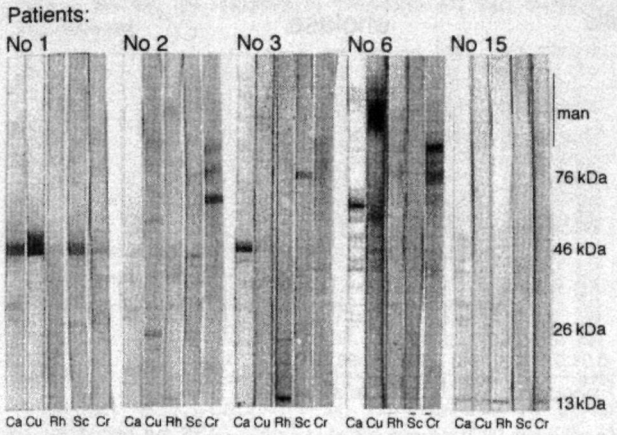


Fig. 2. IgE immunoblotting strips of all five study yeasts incubated in sera of five study patients. Lyophilized antigen load per strip was 0.6 mg for *Candida albicans* (Ca), 0.45 mg for *C. utilis* (Cu), 0.35 mg for *Rhodotorula rubra* (Rh), 0.9 mg for *Saccharomyces cerevisiae* (Sc), and 0.5 mg for *Cryptococcus albidus* (Cr).

C. albicans, against which 19 (95%) and 18 (90%) patients, respectively, had a response. The corresponding patient numbers for IgA class antibodies were 15 (75%) and 17 (85%). Eleven (55%) patients had both IgG and IgA responses to *Cr. albidus*, whereas seven patients (35%) had IgG and five patients (25%) IgA against *R. rubra*. None of the patients had IgA against *C. utilis*, and only four patients (20%) had an IgG response.

Important individual antigens were the high-molecular-weight, mannan-like components, against which 17 (*C. albicans*), 14 (*S. cerevisiae*), 10 (*Cr. albidus*), and seven (*R. rubra*) patients had an IgG response. The corresponding patient numbers in IgA immunoblotting were 17 (*C. albicans*), seven (*S. cerevisiae*), two (*Cr. albidus*), and none (*R. rubra*). Neither IgG nor IgA responses against *C. utilis* mannan were seen in this group. Only occasional IgG responses were seen against yeast proteins except for the 20-kDa (18 patients) and 25-kDa (14 patients) bands of *S. cerevisiae*, a 32-kDa (seven patients) band of *Cr. albidus*, and a 21-kDa (five patients) band of *C. albicans*. An IgA response to the 20-kDa band of *S. cerevisiae* was seen in 12 patients.

Discussion

Although different yeasts have been long known to share common antigens, their significance as allergens has, until recently, been evaluated only by SPT (9, 10, 28). In SPT, the patients react to several yeast extracts simultaneously, a fact which suggests possible allergenic cross-reactivity among the yeast species studied, or that patients have been exposed to all these yeasts. Despite abundant simultaneous skin reactivity, specific IgE antibodies in immunoblotting to the same extent were seen only against *C. albicans* and *C. utilis*. Natural exposure to *C. albicans* occurs, as indicated by the strong IgG response, which correlates with the extent of saprophytic growth; in other words, exposure (32).

The skin reactivity, as well as IgE antibodies, to other yeasts, except *C. albicans*, is probably due to cross-reacting antibodies. This is especially striking for *C. utilis*, which, according to the IgA and IgG

Table 2. IgE binding of 20 studied sera to allergenic bands of five studied yeasts

Mol. wt. (kDa)	*C. albicans*		*C. utilis*		*R. rubra*		*S. cerevisiae*		*Cr. albidus*	
	n[a]	%[b]	*n*	%	*n*	%	*n*	%	*n*	%
150	2	14	–		–		–		–	
95	1	7	–		–		–		1	25
76	–		–		–		2	50	–	
65	–		–		–		–		1	25
63	3	21	–		–		–		–	
57	1	7	1	11	–		–		–	
50	1	7	–		–		–		–	
46	7	50	5	56	1	13	1	25	1	25
43	1	7	1	11	–		1	25	–	
39	1	7	1	11	–		–		–	
37	2	14	2	22	–		–		–	
32	–		2	22	–		–		–	
29	9	64	–		–		–		–	
28	–		–		1	13	1	25	–	
27	–		1	11	–		–		–	
26	–		–		4	50	–		–	
20	–		–		1	13	1	25	–	
13	2	14	2	22	4	50	–		1	25
Total[c]	14		9		8		4		4	

[a]No. of patients with IgE response to band.
[b]Percentage of all patients with IgE response to yeast.
[c]Total number of patients with IgE response to each yeast.

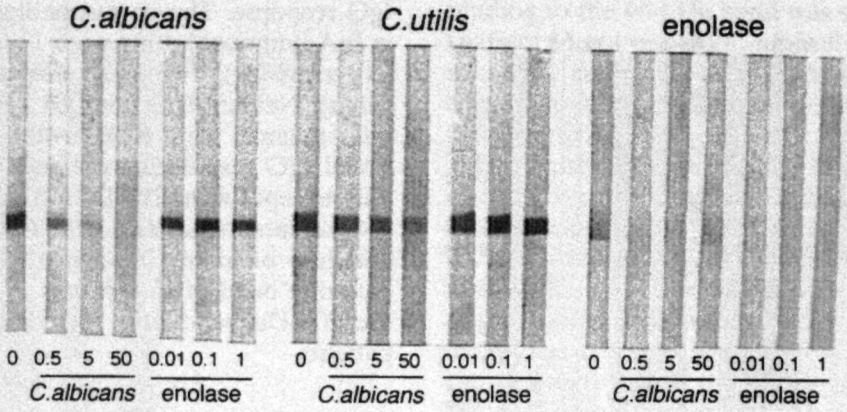


Fig. 3. IgG immunoblotting inhibition of blotted strips of *Candida albicans* (0.3 mg per strip), *C. utilis* (0.38 mg per strip), and purified *Saccharomyces cerevisiae* enolase (0.08 mg per strip), with individual serum from patient 1. Concentrations of liquid phase inhibiting antigens of *C. albicans* and *S. cerevisiae* enolase were mg/ml.

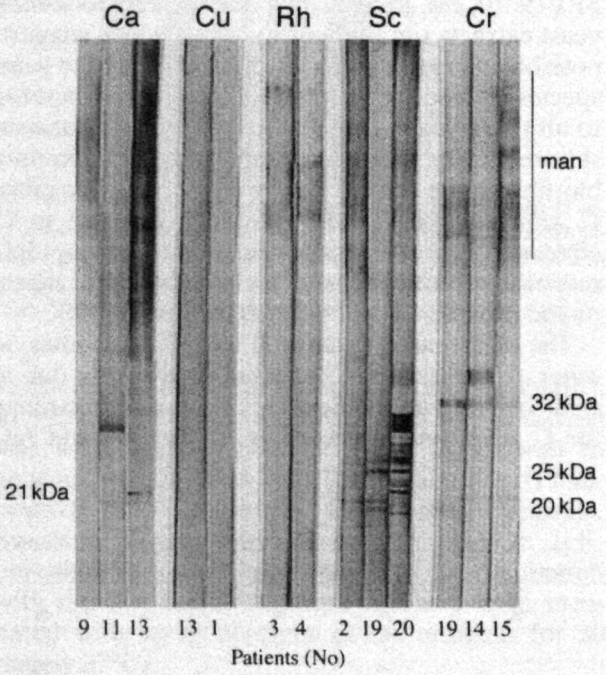


Fig. 4. Examples of IgG immunoblotting with five study yeasts, *Candida albicans* (Ca), *C. utilis* (Cu), *Rhodotorula rubra* (Rh), *Saccharomyces cerevisiae* (Sc), and *Cryptococcus albidus* (Cr). Antigen concentrations of strips were as in Fig. 2. Most important IgG-binding antigens are indicated on left and right.

responses, causes very little exposure, at least as compared to patients exposed to industrial *C. utilis* dust. In the 1970s, emission of *C. utilis* yeast dust of industrial origin caused allergic symptoms in Äänekoski (Finland). In a population of 60 patients from the factory environment, 30% were positive in *C. utilis* IgG immunoblotting and 42% in IgA immunoblotting (results not shown). As exposure to *C. utilis* causes IgG and IgA responses, most of the patients of this study may not have been exposed to *C. utilis*. The patients did not report any history of industrial yeast exposure.

The IgE response to *C. utilis* is probably due to cross-reacting IgE antibodies, since only one out of nine *C. utilis* IgE-positive subjects showed an IgG response to *C. utilis*. This patient also showed the strongest IgE response to *C. utilis*. By using the serum of this patient, the cross-reactivity of the most important antigens, the 46-kDa proteins of *C. albicans* and *C. utilis*, was confirmed. The cross-reactivity appeared to be partial, suggesting that *C. utilis* has its own epitopes for IgE. This finding suggests true exposure to *C. utilis* in this patient in agreement with the *C. utilis*-specific IgG response.

A recent paper showed partial cross-reactivity between purified *C. albicans* and *S. cerevisae* enolases, both with a molecular weight of 46-kDa (21). It was shown that *C. albicans* enolase could inhibit *S. cerevisiae* enolase completely, whereas *S. cerevisiae* enolase could only partially inhibit IgE binding to *C. albicans* enolase. This implies that, in addition to common epitopes, *C. albicans* enolase also has its own unique epitopes. We found the same phenomenon, suggesting the presence not only of cross-reacting epitopes in *C. utilis* and *S. cerevisiae* enolase but also of unique epitopes of the *C. albicans* 46-kDa band. On the other hand, the 46-kDa band could be a complex of several bands with the same molecular weight. However, our results suggest that the 46-kDa bands of *C. albicans* and *C. utilis* are also enolases.

In our SPT survey of the urban population of Äänekoski, the Finnish industrial town exposed to emission of *C. utilis*, skin reactivity to *C. utilis* was found to be equal to that of another industrial urban population in Varkaus, Finland, a town without yeast dust exposure (results not shown). In a

Table 3. IgA and IgG responses to five yeasts and their mannans in immunoblotting

	IgA		IgG	
	Total[a]	Mannan	Total[b]	Mannan
C. albicans	17 (85%)[c]	17 (100%)[d]	18 (90%)	17 (94%)
C. utilis	0 (0%)	0 (0%)	4 (20%)	0 (0%)
R. rubra	5 (25%)	0 (0%)	7 (35%)	7 (100%)
S. cerevisiae	15 (75%)	7 (47%)	19 (95%)	14 (74%)
Cr. albidus	11 (55%)	2 (18%)	11 (55%)	10 (91%)

[a]Total number of IgA- and [b]IgG-positive patients for each yeast.
[c]Percentage of all studied patients.
[d]Percentage of patients with positive yeast-specific response.

further SPT analysis, these populations also revealed skin reactivity to *C. albicans* in equal proportions. These observations also are well explained by the clear cross-reactivity of the 46-kDa main allergens of both *C. albicans* and *C. utilis* and primary sensitization by *C. albicans.*

More frequent IgE responses, as well as IgG and IgA responses, were observed to *C. albicans* than *S. cerevisiae* and the airborne yeasts, except *C. utilis*, suggesting sensitization by saprophytic growth. Having been primarily sensitized by saprophytic exposure, these patients may suffer symptoms when they come into contact with cross-reacting yeasts, such as *Rhodotorula* and *Cryptococcus*, in food or elsewhere in nature. The cross-reactivity of these yeasts may thus explain some of the symptoms found in patients with respiratory, cutaneous, or gastrointestinal allergy.

S. cerevisiae has earlier been connected with urticaria and atopic dermatitis (3, 8). The present study has shown that although frequent exposure to *S. cerevisiae* occurs, according to IgA and IgG antibodies, atopic sensitization (IgE antibodies) is not as frequent as to *C. albicans.* As the 46-kDa enolase allergen of *S. cerevisiae* is labile in solutions, at high temperatures and in the gastrointestinal tract, it is likely that many of the anti-*S. cerevisiae* enolase antibodies are due to primary sensitization by *C. albicans* enolase (33, 34).

The previously reported cross-reactivities of the antimannan IgE antibodies to *C. albicans*, *S. cerevisiae*, and *P. ovale* were not apparent in this population by immunoblotting analysis (23–25). This is due to the low sensitivity of immunoblotting to detect the antimannan antibodies, although slightly elevated mannan RAST values were found. As regards *P. ovale*, it has been shown that mannan-specific IgE antibodies are difficult to distinguish by immunoblotting, because mannan, as a large molecule, hardly enters the SDS–PAGE gel (35).

The cross-reactivity of polysaccharide as well as protein components of various yeasts is becoming better understood. Exposure to yeasts completely different biologically takes very different forms, ranging from saprophytic growth on skin and mucous membranes to airborne exposure and ingested food. The finding that these different yeast species cross-react on an antigenic and allergenic level will help us to understand and explore their sensitizing properties in the future.

Acknowledgments

This study was supported by grants from the Academy of Finland, the Finnish Allergy Research Foundation, the EU Human Capital and Mobility Program, the Ahokas Foundation, and the Cultural Foundation of Finland. J. S. holds an EU Human Capital and Mobility Grant. We thank Mrs Kaarina Jokela and Mrs Leena Kavén-Honka for excellent technical assistance; Prof. Roland Einarsson and Mr Erkki Nieminen for important cooperation.

References

1. Gumowski P, Lech B, Chaves I, Girard JP. Chronic asthma and rhinitis due to *Candida albicans*, *Epidermophyton* and *Trichophyton*. Ann Allergy 1987;59:48-51.
2. Itkin IH, Dennis M. Bronchial hypersensitivity to extract of *Candida albicans*. J Allergy 1966;37:187-94.
3. James J, Warin RP. An assessment of the role of *Candida albicans* and food yeasts in chronic urticaria. Br J Dermatol 1971;84:227-37.
4. Keeney EL. *Candida* asthma. Ann Intern Med 1951;34: 223-6.
5. Savolainen J, Lammintausta K, Kalimo K, Viander M. *Candida albicans* and atopic dermatitis. Clin Exp Allergy 1993;23:332-9.
6. Clemmensen OJ, Hjorth N. Treatment of dermatitis of the head and neck with ketoconazole in patients with type I sensitivity to *Pityrosporum orbiculare*. Semin Dermatol 1983;2:26-9.
7. Kieffer M, Bergbrandt I-M, Færgeman J, et al. Immune reactions to *P. ovale* in adult patients with atopic dermatitis and seborrhoic dermatitis. J Am Acad Dermatol 1990;22: 739-49.
8. Kortekangas-Savolainen O, Lammintausta K, Kalimo K. Skin prick test reactions to brewer's yeast (*Saccharomyces cerevisiae*) in adult atopic dermatitis patients. Allergy 1993;48:147-50.
9. Hasenclever HF, Mitchell WO. Immunochemical studies on polysaccharides of yeasts. J Immunol 1964;93:763-71.
10. Tsuchiya T, Fukazawa Y, Taguchi M, Nakase T, Shinoda T. Serological aspects on yeast classification. Mycopathol Mycol Appl 1974;53:77-91.
11. Doekes G, Kaal MJH, van Ieperen-van Dijk AG. Allergens of *Pityrosporum ovale* and *Candida albicans*. II. Physicochemical characterization. Allergy 1993;48:401-8.
12. Jensen-Jarolim E, Poulsen LK, With H, Kieffer M, Ottevanger V, Skov PS. Atopic dermatitis of the face, scalp and neck: type I reaction to the yeast *Pityrosporum ovale*? J Allergy Clin Immunol 1992;89:44-51.
13. Johansson S, Karlström K. IgE-binding components in *Pityrosporum orbiculare* identified by an immunoblotting technique. Acta Derm Venereol (Stockh) 1991;71:11-16.
14. Kortekangas-Savolainen O, Kalimo K, Lammintausta K, Savolainen J. IgE-binding components of baker's yeast recognized by immunoblotting analysis. Simultaneous IgE binding to mannan and 46-48 kD allergens of *S. cerevisiae* and *C. albicans*. Clin Exp Allergy 1993;23:179-84.
15. Longbottom J, Brighton WD, Edge G, Pepys J. Antibodies mediating type I skin test reactions to polysaccharide and

protein antigens of *Candida albicans*. Clin Allergy 1976;6: 41-9.
16. Nermes M, Savolainen J, Viander M, Lammintausta K, Kalimo K. Determination of IgE antibodies to *Candida albicans* mannan with nitrocellulose-RAST in patients with atopic disease. Clin Exp Allergy 1994;24:318-23.
17. Savolainen J, Viander M, Einarsson R, Koivikko A. Immunoblotting analysis of concanavalin A-isolated allergens of *Candida albicans*. Allergy 1990;45:40-6.
18. Savolainen J, Viander M, Koivikko A. IgE, IgA and IgG antibody responses to carbohydrate and protein antigens of *Candida albicans* in asthmatic children. Allergy 1990;45:54-63.
19. Baldo BA, Baker RS. Inhalant allergies to fungi: reactions to bakers' yeast (*S. cerevisiae*) and identification of bakers' yeast enolase as an important allergen. Int Arch Allergy Appl Immunol 1988;86:201-8.
20. Ishiguro A, Homma M, Torii S, Tanaka K. Identification of *Candida albicans* antigens reactive with immunoglobulin E antibody of human sera. Infect Immun 1992;60:1550-7.
21. Ito K, Ishiguro A, Kanbe T, Tanaka K, Torii S. Detection of IgE antibody against *Candida albicans* enolase and its cross-reactivity to *Saccharomyces cerevisiae* enolase. Clin Exp Allergy 1995;25:522-8.
22. Savolainen J. A standardized densitometric immunoblotting analysis of *Candida albicans* allergens. Clin Exp Allergy 1995;25:357-63.
23. Doekes G, van Ieperen-van Dijk AG. Allergens of *Pityrosporum ovale* and *Candida albicans*. I. Cross-reactivity of IgE-binding components. Allergy 1993;48:394-400.
24. Nermes M, Savolainen J, Kortekangas-Savolainen O. Nitrocellulose-RAST analysis of allergenic crossreactivity in *Candida albicans* and *Saccharomyces cerevisiae* mannans. Int Arch Allergy Immunol 1995;106:118-23.
25. Savolainen J, Broberg A. Allergenic crossreactivity of *Pityrosporum ovale* and *Candida albicans*. Clin Exp Allergy 1992;22:469-74.
26. Cornillon J, Touraine JL, Touraine R. Manifestations asthmatiques probablement liées à une allergie par inhalation de poudre de *Candida tropicalis*. Une nouvelle allergie professionelle. Rev Fr Allergol Immunol 1976;16:289-90.
27. Rantio-Lehtimäki A. Mould spores and yeasts in outdoor air. Allergy 1985;40: Suppl 3:17-20.
28. Koivikko A, Kalimo K, Nieminen E, Savolainen J, Viljanen M, Viander M. Allergenic crossreactivity of yeasts. Allergy 1988;43:192-200.
29. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 1970;227:680-5.
30. Towbin HT, Staehelin T, Gordon J. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc Natl Acad Sci U S A 1979;76:4350-4.
31. Bengtson A, Rolfsen W, Einarsson R. Characterization of allergens and patient sera by a nitrocellulose immunoprint technique. Int Arch Allergy Appl Immunol 1985;78:139-44.
32. Savolainen J, Koivikko A, Kalimo K, Nieminen E, Viander M. IgE, IgA and IgG antibodies and delayed skin response to *Candida albicans* antigens in atopics with and without saprophytic growth. Clin Exp Allergy 1990;20:549-54.
33. Kortekangas-Savolainen O, Savolainen J, Einarsson R. Gastrointestinal stability of baker's yeast allergens studied by *in vitro* analysis. Clin Exp Allergy 1993;23:587-90.
34. Kortekangas-Savolainen O, Einarsson R. Thermal and storage stability of *Saccharomyces cerevisiae* (baker's yeast) allergens. Clin Exp Allergy 1994;24:257-62.
35. Lintu P, Savolainen J, Kalimo K. Immunoblotting and nitrocellulose RAST analysis of mannan and protein allergens of *Pityrosporum ovale*. Clin Exp Allergy 1997;27: 87-95.

JOURNAL OF CLINICAL MICROBIOLOGY, July 1989, p. 1689–1691
0095-1137/89/071689-03$02.00/0

Vol. 27, No. 7

Saccharomyces cerevisiae Pneumonia in a Patient with Acquired Immune Deficiency Syndrome

OSSAMA W. TAWFIK,[1] CHRISTOPHER J. PAPASIAN,[2]* ANITA Y. DIXON,[1,3] AND LONNA M. POTTER[3]

Department of Pathology and Oncology, University of Kansas School of Medicine, Kansas City, Kansas 66103[1]; Department of Pathology, Truman Medical Center—University of Missouri, Kansas City, Missouri 64108[2]; and Laboratory Service, Veterans Administration Medical Center, Kansas City, Missouri 64128[3]

Received 3 January 1989/Accepted 4 April 1989

The clinical course of a patient with a polymicrobial pneumonia that included *Saccharomyces cerevisiae* infection is described. *S. cerevisiae* was recovered from autopsy cultures of the lungs, spleen, oral mucosa, and small intestine, and organisms morphologically consistent with *S. cerevisiae* were visualized in histologic sections of the lung. The role of this organism as a human pathogen is reviewed.

Saccharomyces cerevisiae is frequently referred to as brewers' or bakers' yeast because of its use in the production of beer, wine, and baked goods. It has also been promoted by health food enthusiasts as a nutritional supplement in the form of brewers' yeast tablets or powder containing viable organisms. Consequently, *S. cerevisiae* is a microorganism that we frequently ingest, and yet this yeast has only rarely been associated with serious human infection. We report a case of *S. cerevisiae* pneumonia, with evidence for dissemination, in a patient with the acquired immune deficiency syndrome.

A 39-year-old homosexual male was admitted for evaluation of progressive weakness in his upper and lower extremities, tingling in the digits of his hands and feet, and lower-back pain. These symptoms were first noted approximately 6 months prior to admission. His medical history was significant for human immunodeficiency virus infection (first documented 17 months prior to admission), hepatitis B infection, herpes esophagitis, oral and esophageal candidiasis, *Staphylococcus aureus* urinary tract infection and septicemia, arthritis, gout, hypertension, irreversible neurological deficit (secondary to human immunodeficiency virus infection), right inguinal hernia repair, and right hiatal hernia repair.

On physical examination, the temperature, pulse, respirations, and blood pressure were 99.4°F (37.4°C) 48/min, 20/min, and 100/70 mm Hg, respectively; noteworthy physical findings included peripheral neuropathy, dementia, myelopathy, and absence of abnormalities on auscultation of the chest. Remarkable laboratory findings included leukopenia with a left shift (2,500 leukocytes per mm^3, 75% polymorphonuclear neutrophils, 17% bands, 2% lymphocytes, 1% eosinophils, 2% monocytes, and 3% atypical lymphocytes); sodium, 134 meq/liter; calcium, 8.4 mg/dl; albumin, 3.2 g/dl; alkaline phosphatase, 244 IU/liter; aspartate aminotransferase, 107 IU/liter; and lactate dehydrogenase, 419 IU/liter. Roentgenograms of the chest taken on admission revealed no distinct abnormalities. Blood and urine specimens were collected for bacterial culture (and subsequently found to be negative), and supportive therapy with intravenous fluids was initiated. Empiric antimicrobial therapy (ciprofloxacin, 750 mg orally twice a day) was also initiated. At 2 days after admission, the patient became markedly febrile (peak temperature of 104.2°F [40.1°C] 3 days after admission), and 6 days after admission, chest X rays revealed diffuse interstitial infiltrates in both lung fields. Blood gases 6 days after admission were characterized by the following: pH, 7.47; pCO_2, 27 mm Hg; and pO_2, 28 mm Hg. Respiratory secretions were not submitted for fungal or mycobacterial cultures at this time. Oxygen and intravenous pentamidine therapy (200 mg once daily) were initiated, but the patient died 7 days after admission.

Gross findings at an autopsy limited to the trunk revealed diffuse and patchy areas of consolidation of both lungs and bilateral pleural effusions with 200 and 100 ml of pleural fluid in the right and left pleural cavities, respectively. Microscopic examination revealed an intra-alveolar frothy exudate with hyaline membranes, alveolar damage, and bronchial cell hyperplasia with luminal exudate. Fungal cultures of tissue collected at autopsy were incubated in an air atmosphere at room temperature following inoculation onto yeast extract phosphate agar, Sabouraud dextrose agar with and without chloramphenicol and gentamicin, and Sabouraud dextrose agar with chloramphenicol and cyclohexamide; cultures for mycobacteria were inoculated onto Lowenstein-Jensen and Middlebrook 7H11 media and incubated at 35°C in an atmosphere of 5% carbon dioxide and 95% air. Autopsy lung cultures yielded *Mycobacterium avium-M. intracellulare* and *S. cerevisiae* on all inoculated mycobacterial and fungal media, respectively, except Sabouraud dextrose agar with chloramphenicol and cyclohexamide. *Pneumocystis carinii* and yeast forms morphologically consistent with *S. cerevisiae* were seen in histologic sections of lung tissue stained with Grocott methenamine silver stain (Fig. 1); acid-fast bacilli were seen in the same sections stained with Night Blue. *S. cerevisiae* was identified by means of ascospore production and carbohydrate assimilation tests (API 20C clinical yeast systems from Analytab Products and yeast identification cartridge from Abbott Diagnostics).

The spleen and lymph nodes were markedly depleted of lymphoid elements, and there was evidence of extramedullary hematopoiesis in the spleen. Acid-fast bacilli were visualized in both spleen and lymph nodes with a Night Blue stain, but no yeast cells were found with Grocott methenamine silver stains. Cultures of splenic tissue, however, revealed both *M. avium-M. intracellulare* and *S. cerevisiae* on all inoculated mycobacterial and fungal media, respectively, except Sabouraud dextrose agar with chloramphenicol and cyclohexamide. Other remarkable autopsy findings included an occlusive organizing thrombus in the right

* Corresponding author.

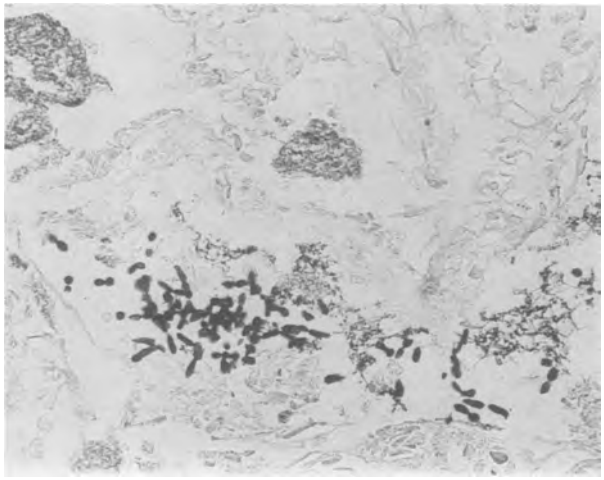


FIG. 1. Histologic section of lung showing yeast cells which morphologically resemble *S. cerevisiae* (Grocott methenamine silver stain). Magnification, ×208.

coronary artery, microscopic evidence of a recent myocardial infarct with reactive pericarditis in the posterior wall of the left ventricle, and approximately 100 ml of serous pericardial effusion. It was concluded that the patient probably died of cardiac arrest secondary to an acute myocardial infarct and respiratory failure due to severe bilateral pneumonia. Additional relevant positive autopsy cultures included recovery of *M. avium-M. intracellulare* from the small intestine, oral cavity, bile, and pleura and recovery of *S. cerevisiae* from the oral cavity and small intestinal tissue. Blood was not submitted for fungal or mycobacterial culture at autopsy.

S. cerevisiae is an ascospore-producing yeast that is an occasional commensal on human mucosal surfaces and is not uncommon in clinical specimens; it is, however, rarely associated with serious human infections (4, 7). A literature review revealed eight cases of potentially serious *Saccharomyces* infections including six fungemias, one peritonitis, and one pleural effusion (Table 1). Although three of the eight patients died, *Saccharomyces* infection was not the primary cause of death in two of these cases (one patient died of complications of disseminated intravascular coagulopathy, and the other died of an insulin reaction); the contribution of *Saccharomyces* infection to the death of the third patient cannot be assessed because he died of respiratory failure and had a polymicrobial pleural effusion that included *S. cerevisiae*, *Escherichia coli*, *S. aureus*, a *Streptococcus* sp., and a *Lactobacillus* sp. (2–4). Infections in the surviving patients responded promptly to antifungal chemotherapy and produced little residual morbidity. *Saccharomyces* spp., including *S. cerevisiae*, have also been associated with genitourinary infections in both males and females as well as mild gastrointestinal and respiratory infections (1, 4, 8, 12).

The case we report is noteworthy because our patient clearly had involvement of multiple visceral organs as indicated by positive autopsy cultures of the oral mucosa,

TABLE 1. Characteristics of patients with serious, culture-proven *Saccharomyces* infections[a,b]

Age (yr) and sex	Predisposing condition	Positive culture site	Leukopenia	Prior antibiotics	Antifungal therapy	Outcome	Comment	Reference
61 (M)	Renal failure; hemodialysis	Blood; central catheter access site	No	Yes	Miconazole flucytosine	Death	Patient died from complications of DIC; autopsy failed to reveal nidus of fungal infection	2
66 (M)	Pancreatic cancer; laparotomy	Peritoneal fluid	No	Yes	Ketoconazole	Death	Death due to insulin reaction; patient failed to complete antifungal therapy	3
52 (M)	COPD; steroids; i.v. drug use; ingestion of brewers' yeast	Pleural fluid	Unk	No	Ketoconazole	Death	*Streptococcus aureus*, *Streptococcus* sp., *Escherichia coli*, and *Lactobacillus* sp. also recovered from pleural fluid	4
66 (M)	Burns; upper GI hemorrhage; mechanical aspiration	Blood	Yes	Yes	Amphotericin B followed by oral nystatin	Cure	Yeast cells observed in histologic sections of esophageal biopsy	5
68 (M)	Ingestion of brewers' yeast	Blood, bone marrow; urine	No	Yes	None	Cure	Discontinuation of brewers' yeast ingestion produced cure	6
38 (M)	Prosthetic tricuspid valve; i.v. drug use	Blood	Unk	Yes	Amphotericin B	Cure	Probable *Saccharomyces* endocarditis; patient died of bacterial endocarditis 8 mo after completing antifungal therapy	9
37 (F)	AIDS; i.v. drug use; renal failure; peritoneal dialysis	Blood	Unk	Yes	Amphotericin B	Cure	Ophthalmologic exam revealed frothy yellow exudates and chorioretinal necrosis	10
54 (F)	Prosthetic mitral valve	Blood; urine	No	Yes	Amphotericin B	Cure	Probable *Saccharomyces* endocarditis	11

[a] The species was determined to be *S. cerevisiae* in all studies except one (11), in which it was undetermined.
[b] Abbreviations: M, male; F, female; DIC, disseminated intravascular coagulopathy; COPD, chronic obstructive pulmonary disease; i.v., intravenous; Unk, unknown; GI, gastrointestinal; AIDS, acquired immune deficiency syndrome.

intestine, spleen, and lungs and by visualization of yeast cells morphologically consistent with *S. cerevisiae* in histologic sections of the lung. We postulate that *S. cerevisiae* colonized the oropharynx of the patient, was subsequently aspirated into the lungs, and disseminated hematogenously to the spleen. Although our patient clearly had *S. cerevisiae* pneumonia, the contribution of this organism to the morbidity and mortality of the patient cannot be assessed because of concurrent *P. carinii* and *M. avium-M. intracellulare* pneumonia. Multiple organ invasion by this relatively benign organism underscores the profound immunologic incompetence induced by human immunodeficiency virus infection.

LITERATURE CITED

1. **Ahnlund, H. O., B. Pallin, R. Peterhoff, and J. Schonebeck.** 1967. Mycosis of the stomach. Acta Chir. Scand. **133**:555–562.
2. **Cimolai, N., M. J. Gill, and D. Church.** 1987. *Saccharomyces cerevisiae* fungemia: case report and review of the literature. Diagn. Microbiol. Infect. Dis. **8**:113–117.
3. **Dougherty, S. H., and R. L. Simmons.** 1982. Postoperative peritonitis caused by *Saccharomyces cerevisiae*. Arch. Surg. **117**:248–249.
4. **Eng, R. H., R. Drehmel, S. M. Smith, and E. J. C. Goldstein.** 1984. *Saccharomyces cerevisiae* infections in man. Sabouraudia **22**:403–407.
5. **Eschete, M. L., and B. C. West.** 1980. *Saccharomyces cerevisiae* septicemia. Arch. Intern. Med. **140**:1539.
6. **Jensen, D. P., and D. L. Smith.** 1976. Fever of unknown origin secondary to brewer's yeast ingestion. Arch. Intern. Med. **136**:332–333.
7. **Kiehn, T. E., F. F. Edwards, and D. Armstrong.** 1980. The prevalance of yeasts in clinical specimens from cancer patients. Am. J. Clin. Pathol. **73**:518–521.
8. **Reiersol, S., and J. Hoel.** 1958. *Saccharomyces carlsbergensis*, possibly a pathogenic. Acta Pathol. Microbiol. Scand. **44**: 313–318.
9. **Rubinstein, E., E. R. Noriega, M. S. Simberkoff, R. Holzman, and J. J. Rahal.** 1975. Fungal endocarditis: analysis of 24 cases and review of the literature. Medicine **54**:331–344.
10. **Sethi, N., and W. Mandell.** 1988. *Saccharomyces* fungemia in a patient with AIDS. N.Y. State J. Med. **88**:278–279.
11. **Stein, P. D., A. T. Folkens, and K. A. Hruska.** 1970. *Saccharomyces* fungemia. Chest **58**:173–175.
12. **Wilson, J. D., B. M. Jones, and G. M. Kinghorn.** 1988. Bread making as a source of vaginal infection with *Saccharomyces cerevisiae*. Sex. Transm. Dis. **15**:35–36.