

IgE-binding components of baker's yeast (*Saccharomyces cerevisiae*) recognized by immunoblotting analysis. Simultaneous IgE binding to mannan and 46–48 kD allergens of *Saccharomyces cerevisiae* and *Candida albicans*

O. KORTEKANGAS-SAVOLAINEN*†, K. KALIMO*, K. LAMMINTAUSTA* and J. SAVOLAINEN†‡

Departments of *Dermatology, †Medical Microbiology and ‡Clinical Allergology, University of Turku, Finland

Summary

The *Saccharomyces cerevisiae* allergens were characterized by IgE-immunoblotting with serum samples of 83 patients; 63 represented patients with atopic dermatitis with previous positive skin prick test or RAST for *S. cerevisiae*, seven patients with AD but negative test results and 13 were non-atopic controls. Disrupted whole body extract of *S. cerevisiae* was used in the assays. From the patients tested 41 patients with atopic dermatitis appeared positive in IgE immunoblotting revealing 22 IgE stained bands. From these bands 10 represented intermediate allergens, and 12 minor allergens. The most frequent staining was obtained with the 48 kD band (39%). When the staining pattern of 45 kD and 48 kD bands and mannan was compared with *Candida albicans* allergens or purified baker's yeast enolase a simultaneous binding was seen with the 48 kD band of *S. cerevisiae* and the 46 kD band of *C. albicans* and enolase whereas the 45 kD band was neither associated with the 46 kD band of *C. albicans* nor purified enolase. High molecular weight staining was found in five samples. The staining pattern was associated with the mannose containing structures in parallel with *C. albicans*.

Clinical and Experimental Allergy, Vol. 23, pp. 179–184. Submitted 28 April 1992; revised 27 July 1992; accepted 3 August 1992.

Introduction

Saccharomyces cerevisiae (*S. cerevisiae*, baker's yeast) is common in our everyday nutrition. In earlier clinical studies it has been suspected to be a triggering factor in chronic urticaria [1] and in bakers' asthma [2]. Positive skin-prick test results also suggest an association with exacerbation of atopic dermatitis (AD) [3].

The knowledge concerning the allergenic properties of *S. cerevisiae* has been limited; only Baldo *et al.* have carried out an immunoblotting study with three patients and suggested that the 51 kD protein, close to the 52 kD *S. cerevisiae* enolase, would be a major allergen [2].

To evaluate the allergenic properties of *S. cerevisiae* more detailed studies including characterization of the extract are needed. In this study patients allergic to *S. cerevisiae*, proven by RAST and skin-prick test (SPT)

techniques, were selected for analysis of the allergenic composition of baker's yeast by IgE immunoblotting technique.

Materials and methods

Preparation of antigens

Source material *S. cerevisiae* was obtained from Oy Alko Ab as a commercial product for household use. According to the manufacturer the yeast had been cultured on a fermentation media, rich in nitrogen and phosphate, and containing beet molasses as described earlier [4].

Crude antigen The crude cytoplasmic extract of the yeast cells was prepared after disruption in an X-press. Briefly, the yeast cells were suspended in 0.125 M ammoniumhydrogencarbonate (50% w/v) and broken in a X-press chamber (Biox Ab, Järfälla, Sweden) with six

Correspondence: Dr O. Kortekangas-Savolainen, Department of Dermatology, University of Turku, 20520 Turku, Finland.

dehydrogenase [16]. It is noteworthy that in our material a 39 kD band was shown to be an intermediate allergen of *S. cerevisiae*.

A diffuse high molecular weight stain was seen in IgE-immunoblots of five patients. With *C. albicans* this kind of stain has been shown to be due to IgE-binding to mannan polysaccharide [5]. As the cell wall of *S. cerevisiae* contains mannan [7], purified mannan and mannan-free proteins of *S. cerevisiae* as well as *C. albicans* were analysed with high molecular weight reactive sera. The high molecular weight stain was associated with purified mannan preparations of *S. cerevisiae* as well as *C. albicans*. Mannan might thus be an important crossreacting component of allergenic yeasts. High molecular weight polysaccharides have been speculated to lie behind allergenic crossreactivity of *Pityrosporum ovale* and *C. albicans* [17]. RAST-inhibition studies with purified polysaccharides from all three yeasts are needed and may clarify the subject.

The connection of yeasts with pathogenesis of other diseases has been noted. Recent studies with Morbus Crohn patients have revealed elevated IgA and IgG responses against baker's yeast antigens [18]. In detailed analysis the important antigen for this response was shown to be a high molecular weight glycoprotein [19]. The relationship of this glycoprotein and the mannan observed in our studies should be explored as they may represent the same antigen.

To analyse further the significance of yeasts as ethio-pathogenetic agents in the disease processes more should be known about the stability of the yeast allergens in food processing and in the gastrointestinal tract. These studies, exploring the detailed allergenic pattern of *S. cerevisiae*, provide the basis for further studies within the field of clinical and experimental allergy.

Acknowledgments

Mrs Leena Kaven-Honka is acknowledged for excellent technical assistance. We thank Professor Roland Einarsson, Pharmacia, Sweden, for valuable support and Assistant Professor Hannu Kalimo for crucial help. This study was supported by grants from the Emil Aaltonen Foundation and the Ida Montin Foundation.

References

- 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.
- Baldo BA, Baker RS. Inhalant allergies to fungi: reactions to baker's yeast (*Saccharomyces cerevisiae*) and identification of baker's yeast enolase as an important allergen. *Int Arch Allergy Appl Immunol* 1988; 86:201-8.
- Kortekangas-Savolainen O, Lammintausta K, Kalimo K. Skin prick test reactions to Baker's yeast in adult atopic dermatitis patients. *Allergy*, in press.
- Suomalainen H, Oura E. Changes in the decarboxylase activity of baker's yeast during the growth phase. *Biochim Biophys Acta* 1959; 31:115-24.
- Savolainen J, Viander M, Einarsson R, Koivikko A. Immunoblotting analysis of concanavalin A-isolated allergens of *Candida albicans*. *Allergy* 1990; 45:40-6.
- Savolainen J, Viander M, Koivikko A. Distribution of water soluble antigens and allergens of *Candida albicans* in blastospore cell extract fractions. *Allergy* 1990; 45:47-53.
- Peat S, Whelan WJ, Edwards TE. Polysaccharides of baker's yeast. Part IV. Mannan. *J Chem Soc* 1961; 1:29-34.
- Hanifin J, Rajka G. Diagnostic features of atopic dermatitis. *Acta Derm Venereol (Stockh)* 1980; 92 (supl.):42-7.
- Aas K. Clinical and experimental aspects of standardization and purification of allergen. *Int Arch Allergy Appl Immunol* 1975; 49:44-54.
- Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 1970; 227:680-5.
- Towbin H, Staehelin T, Gordon J. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc Natl Acad Sci* 1979; 76:4350-4.
- Bengtsson 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.
- 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.
- Iida H, Yahara I. Yeast heat-shock protein of M_r 48 000 is an isoprotein of enolase. *Nature* 1985; 310:688-90.
- Shen HD, Choo KB, Tang RB, Lee CF, Yeh JY, Han SH. Allergenic components of *Candida albicans* identified by immunoblot analysis. *Clin Exp Allergy* 1989; 19:191-6.
- Shen HD, Choo KB, Lee HH, Hsieh JC, Lin WL, Lee WR, Han SH. The 40-kilodalton allergen of *Candida albicans* is an alcohol dehydrogenase: molecular cloning and immunological analysis using monoclonal antibodies. *Clin Exp Allergy* 1991; 21:675-81.
- Savolainen J, Broberg A. Crossreacting IgE-antibodies to *Pityrosporum ovale* and *Candida albicans* in atopic children. *Clin Exp Allergy* 1992; 22:469-74.
- 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.
- Heelan BT, Allan S, Barnes RMR. Identification of a 200-kDa glycoprotein antigen of *Saccharomyces cerevisiae*. *Immunol Lett* 1991; 28:181-6.