

IgG and IgE immune response against the surface glycoprotein gp200 of *Saccharomyces cerevisiae* in patients with atopic dermatitis *

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Abstract

The heat-stable and soluble glycoprotein gp200 (molecular weight 200 kDa) is part of the cell wall of *S. cerevisiae*. Recently, an association was shown between IgA and IgG against gp200 and inflammation in Crohn's disease. Gp200 is able to induce a proliferation of human lymphocytes *in vitro*, together with a natural killer cell associated cytotoxicity. Specific IgE against *Saccharomyces cerevisiae* (baker's or brewer's yeast) may be detected in approximately 73%, against *Candida albicans* in 68% of those patients suffering from severe atopic dermatitis. The aim of this study was to elucidate the possible role of an anti-gp200 immune response for the pathogenesis of atopic dermatitis by immunoblot analysis.

Anti-gp200 IgE was found in 55% of healthy individuals, in 67% of individuals with atopic predisposition without eczema, in 63% of the patients with mild atopic dermatitis, and in 86% of patients with severe atopic dermatitis, respectively. On the contrary, anti-gp200 IgG could be shown in 55% of healthy individuals, in 89% of individuals with atopic predisposition but without eczema, in 100% of patients with mild atopic dermatitis, and in 79% with severe atopic dermatitis, respectively. No immunoreactivity was found when an extract of *Arxula adenivorans* was used as antigen. These results underline the specificity of the immunoblot results with gp200 from *Saccharomyces cerevisiae*. It can be concluded that occurrence of specific IgE against *Saccharomyces cerevisiae* cannot be explained by a cross reactivity, e.g., against *Candida albicans* allergens. Further investigations with the recombinant gp200 will give information on the role of this glycoprotein both in atopic dermatitis and Morbus Crohn.

Key words: allergy, atopic dermatitis, glycoproteins, humoral immune response, immunoblot, IgE antibodies, *Saccharomyces cerevisiae*

Introduction

The heat-stable and soluble glycoprotein gp200 with an apparent molecular weight of 200 kDa represents an antigen of the cell wall of *Saccharomyces (S.) cerevisiae*, which was first identified in 1991 by Heelan et al. [11]. SDS-PAGE (sodiumdodecylsulfate-polyacrylamid gel electrophoresis) analysis of the soluble protein extract of *S. cerevisiae* revealed a single predominant moiety of an apparent size of 200 kDa which was strongly periodic acid Schiff-positive. In addition, a periodate treatment of the strips reduced

the binding activity of the *Saccharomyces*-antibodies (from rabbit) caused by oxidizing the carbohydrate part by increasing the concentration. The resulting protein contains a high amount of glucose, which is responsible for antigenicity.

The specific immune response against gp200 was proved by using Crohn's disease sera in immunoblot analysis (SDS-PAGE according to Laemmli [17]) with increased IgA and IgG levels against *S. cerevisiae*, determined by the enzyme-linked immunosorbent assay (ELISA), as described by Barnes et al. [2]. All sera of 8 patients with Crohn's disease showed positive reactions to the 200 kDa band both for IgA and IgG, whereas healthy controls were negative.

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It is known that the cell wall of *S. cerevisiae* represents a layered structure consisting of β -glucane and 10% mannose proteins [1]. It is assumed that gp200 is a considerable part of the yeast cell wall glycoproteins, which contain a complex of O- and N-linked oligomannose. McKenzie et al. [18] and Barnes et al. [2] demonstrated a correlation between increased anti-*Saccharomyces*-IgA levels and Crohn's disease.

Atopic dermatitis (AD) is a skin disease, which is influenced by environmental and genetic factors. Recent studies pointed out that yeasts, especially *Malassezia (M.) furfur* (*Pityrosporum ovale*) [26, 28] and *Candida (C.) albicans* [19, 25] may also contribute to aggravate AD.

The investigations of Darroch et al. [4] showed that gp200 is able to induce proliferation of human lymphocytes *in vitro*, together with a natural killer cell associated cytotoxicity. The aim of this study was to elucidate the possible role of the anti-gp200 immune response to the pathogenesis of AD.

Material and methods

Patients and control groups

Altogether 42 individuals divided into 4 groups were examined:

Group 1	Healthy individuals	$n = 11$
Group 2	Healthy individuals with atopic predisposition	$n = 9$
Group 3	Patients with slight AD	$n = 8$
Group 4	Patients with severe AD	$n = 14$

Eighteen of the 22 patients with AD were outpatients, and four were inpatients of the Department of Dermatology of the University of Leipzig. The atopic criteria according to Hanifin and Rajka [10] were applied, and predisposition for atopic skin diseases was confirmed by the "Erlanger Atopietest" according to Diepgen et al. [5]. Since there were great differences regarding the severity of the disease the patients were divided into a group with slight and a second group with severe skin appearances, according to the SCORAD index [7].

The control group also had to be divided into two groups, because among them were individuals with a predisposition for an atopic disease. In the group of patients with atopic predisposition there were subjects without any skin lesions, but who had reacted to at least two allergens in the prick test. When compared to the healthy individuals this group showed

significantly higher total IgE levels ($p = 0.022$; u-test, following Mann & Whitney, StatWorks, Apple Macintosh), but nevertheless the "Erlanger Atopietest" was still negative, except in one proband.

SDS-PAGE and immunoblotting

The separation of the crude extract of gp200 (prepared according to Young et al. [29] from the yeast *S. cerevisiae* strain J. Sainsbury PLC, London) was carried out using the electrophoresis apparatus from Bio Rad, as described by Laemmli [16] with a 5% stacking gel and a 7.5% resolving gel. The sample contained $1.5 \mu\text{g}/\mu\text{L}$ protein, and had been mixed in a proportion of 1:1 with a sample buffer containing 5% 2-mercaptoethanol and 25% glycerol which had been heated at 95°C for 5 min prior to loading on the gel. Molecular weight standard (6 μL) was used as control (HMW-SDS Calibration Kit for molecular weight determination using electrophoresis, Pharmacia Biotech, Freiburg, Germany). After the protein transfer to nitrocellulose membrane (Optitran BA-S 83, cellulose nitrate membrane, $0.2 \mu\text{m}$, Schleicher & Schuell, Dassel, Germany) as described by Towbin et al. [27], the unspecific binding was blocked by incubation for 30 min at room temperature in TBST buffer (150 mM NaCl, 20 mM Tris-HCl, 0.4% Tween 20, pH 7.5). Incubation was performed overnight at 4°C with the sera diluted in TBST buffer (0.1% Tween 20). Protein bands were identified using monoclonal antibodies (monoclonal anti-human IgE, anti-mouse IgG, whole molecule, alkaline phosphatase), anti-human IgG (whole molecule, alkaline phosphatase, Sigma, Deisenhofen, Germany). Blots were stained by Western blue stabilized substrate for alkaline phosphatase (Promega, Heidelberg, Germany).

The monoclonal antibody against gp200 (mab 92-276/018) [3] was used as a positive control. The molecular weight standard was stained with Ponceau red.

Determination of IgE

Both total and the antigen-specific IgE antibodies were analyzed by fluorescence immunoassay (UniCAP Total IgE and UniCAP Specific IgE, Uppsala, Sweden). In order to detect specific IgE antibodies directed against *S. cerevisiae* gp200, crude extract was covalently coupled to immunoCAP. This procedure was kindly done by Pharmacia & Upjohn Company in Uppsala, Sweden. UniCAP specific IgE against *C. albicans* mannoprotein and *S. cerevisiae* mannan were

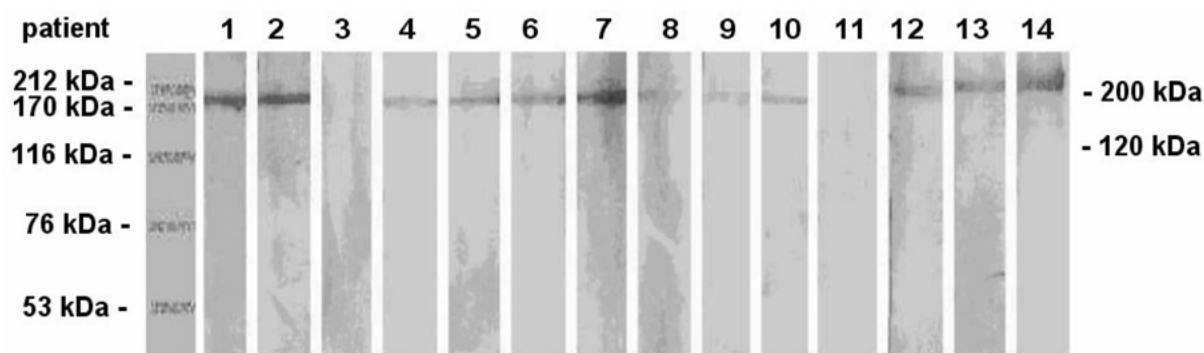


Figure 1. Immunoblot analysis of IgE against gp200 of *S. cerevisiae* in patients with severe AD. 12 of 14 sera from these patients (group 4) were reactive for gp200.

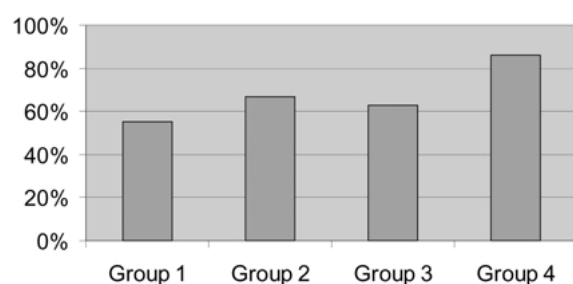


Figure 2. Frequency of anti-IgE against gp200 of *S. cerevisiae*. Group 1, healthy persons: 55%; Group 2, healthy persons with atopic tendency: 67%; Group 3, patients with slight AD: 63%; Group 4, patients with severe AD: 86%.

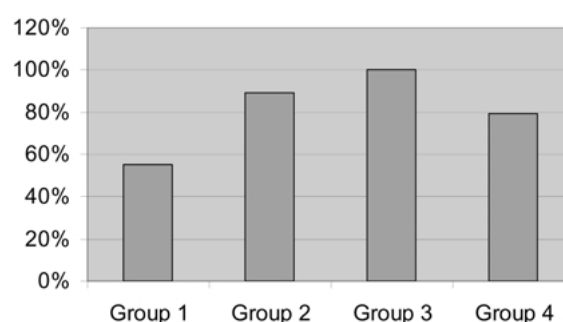


Figure 3. Frequency of anti-IgG against gp200 of *S. cerevisiae*. Group 1, healthy persons: 55%; Group 2, healthy persons with atopic tendency: 89%; Group 3, patients with slight AD: 100%; Group 4, patients with severe AD: 79%.

provided by Pharmacia & Upjohn. Significance testing was done using the u test according to Mann & Whitney.

Results

Detection of IgE against gp200

Seven out of eleven healthy individuals within the first group were positive for IgE against gp200 in immunoblot analysis. In the second group with healthy persons with atopic predisposition, 6 out of 9 individuals were positive for anti-gp200 IgE. 5 out of 8 sera of patients with slight AD (group no. 3) showed anti-IgE against gp200 of this yeast extract protein from *S. cerevisiae*. Within the group 4, sera from 12 of 14 patients with severe AD were positive for anti-gp200 IgE antibodies (Figures 1 and 2). No immunoreactivity was found when an extract of *Arxula adeninivorans* was used as antigen which underlines the specificity of the immunoblot results with gp200 from *S. cerevisiae*.

Detection of IgG against gp200

In the first group with healthy individuals IgG antibodies against gp200 were detected in six out of 11 individuals. In the second group with healthy individuals, but with predisposition for atopy, eight out of 9 individuals had strong IgG reactivity against gp200 in immunoblot. All patients with slight AD showed a reaction in immunoblots. 10 out of 14 sera of patients with severe AD showed an IgG response directed against gp200.

There was a clear correlation between IgG binding within the several groups with lowest frequency in the first and highest IgG reactivity in the third group. Surprisingly, the fourth group showed again a slight decrease in the frequency of reactions (Figure 3). Again, no immunoreactivity was found with the extract of *Arxula adeninivorans* as antigen.

Determination of total IgE

Using fluorescence immunoassay UniCAP analysis, total IgE level in the serum of 42 individuals was determined. A significantly increased total IgE level could be demonstrated in AD when compared to healthy persons. Total IgE levels significantly increased with severity of AD from group 1 up to group 4 (Figure 4). The concentrations were: group 1: 27.44 kU l⁻¹; group 2: 137.77 kU l⁻¹; group 3: 487.18 kU l⁻¹, and group 4: 4754.87 kU l⁻¹. A highly significant difference ($p < 0.0001$, u test according to Mann & Whitney) could be demonstrated between groups 1/3, 1/4 and 2/4. Between group 1 and 2 there was no significant difference, thus the comparison of group 3 and 4 again revealed high significance with $p < 0.002$.

Determination of gp200-specific IgE

Specific IgE against gp200 of *S. cerevisiae* was determined by UniCAP fluorescence immunoassay (Pharmacia & Upjohn, Uppsala, Sweden). In addition, specific IgE against mannoprotein of *C. albicans* and mannan of *S. cerevisiae* was analyzed. Patients from group 1 and 2 had no detectable specific IgE against these yeast proteins. However, both patients with slight and severe AD exhibited highly significant levels of specific IgE antibodies against all three protein specificities of the two yeast species (Table 1). Within the distinct groups 1 to 4 no significant differences were found between the three different IgE specificities. However, when *S. cerevisiae* mannan was compared between group 3 and 4 a significant difference was found ($p < 0.01$). In addition, *C. albicans* mannoprotein specific IgE significantly increased from group 3 to 4 ($p < 0.0001$). Interestingly, the increase of *S. cerevisiae* gp200 IgE from group 3 to 4 was also highly significant ($p < 0.0001$).

Discussion

The ascomycetous yeast *S. cerevisiae* is a single cell organism which is found in the environment, especially on various plants, on fruit, and in soil. Human beings are exposed to antigens of this yeast via food-stuffs because of the occurrence of *S. cerevisiae* in its living form but also after cooking or heating.

McKenzie et al. [18] and Barnes et al. [2] detected an association between increased anti-Saccharomyces-IgA levels and inflammatory bowel diseases, in particular Crohn's disease. The *S.*

Table 1. Specific IgE levels against *S. cerevisiae* mannan and gp200 in comparison to *C. albicans* mannoprotein IgE determined by fluorescence immunoassay

	Specific IgE [U/ml, x ± SEM] against		
	<i>S. cerevisiae</i> mannan	<i>C. albicans</i> mannoproteins	<i>S. cerevisiae</i> gp200
Group 1 (n = 11)	<0.35	<0.35	<0.35
Group 2 (n = 9)	<0.35	<0.35	<0.35
Group 3 (n = 8)	0.36 ± 0.02	0.39 ± 0.06	0.35
Group 4 (n = 14)	5.66 ± 6.44	7.08 ± 7.88	5.21 ± 5.66
Significance (comparison of group 1, 2, 3 to 4)	$p < 0.01$	$p < 0.0001$	$p < 0.0001$

cerevisiae antibodies were different from *C. albicans* antibodies, which are detected to a similar extent in Crohn's disease, Colitis ulcerosa, but also in healthy individuals [9, 18].

In preliminary studies it was confirmed that there is a correlation between the severity of AD and the serum level of total IgE. Zschiesche et al. [30] demonstrated that patients with slight symptoms exhibited total IgE level of 144.3 ± 41.1 kU l⁻¹ which was significantly lower than the mean value of 2162.8 ± 230.4 kU l⁻¹ in patients with severe AD. In addition, the same authors demonstrated that specific IgE against *S. cerevisiae* was found in 72% of the patients suffering from severe AD, in 68% of these patients IgE against *C. albicans* was found, and in 14% of patients against *M. furfur*. These authors suggested that specific IgE to *C. albicans* and *S. cerevisiae* is probably an indication of polysensibilization. Our study also showed significantly increased total IgE levels in AD (Figure 3). Especially, those patients with severe AD (group 4) had an excessively elevated total IgE of approximately 4754 kU l⁻¹.

Sander et al. [20] examined the humoral immune response to gp200 of baker's yeast. Contrary to Heelan et al. [11] Sander et al. surprisingly showed that antibodies were also found in healthy controls. Young et al. [29] undertook further characterization of the gp200. Using the size exclusion chromatography of the extract from *S. cerevisiae*, they discovered a high molecular glycoprotein antigen. This glycoprotein antigen (Sc 500) was shown to be identical with gp200.

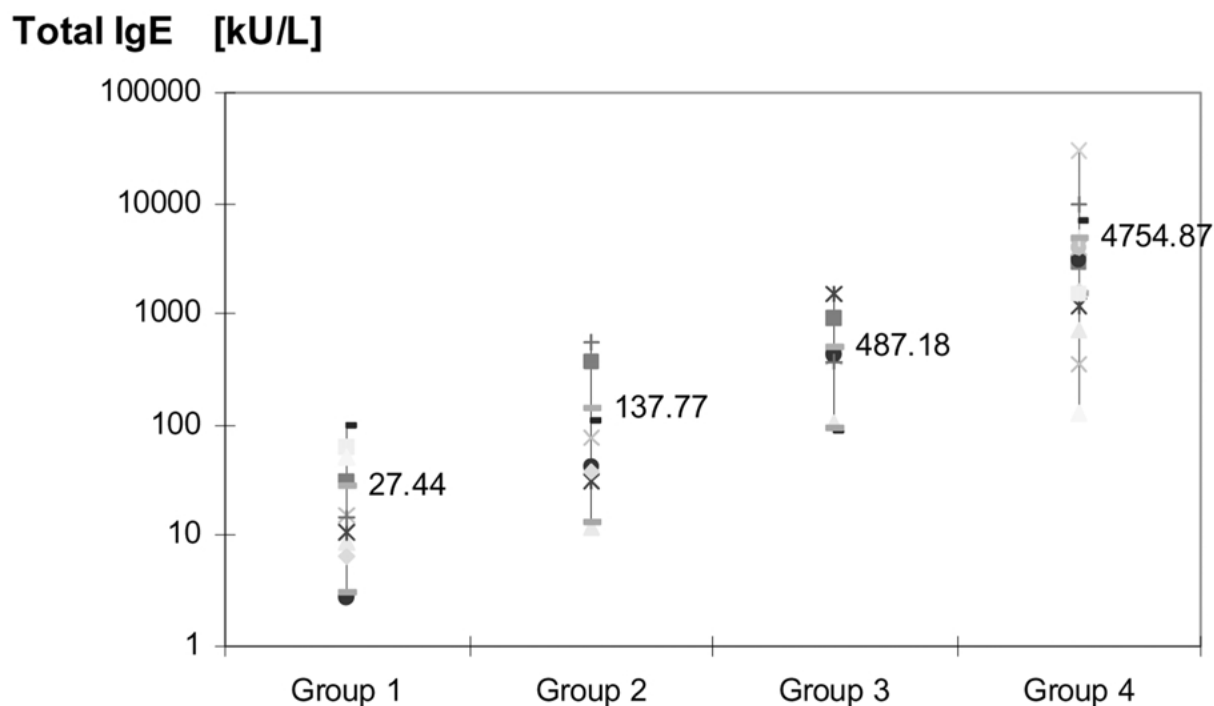


Figure 4. ImmunoCAP analysis of total IgE serum levels in 42 individuals. The geometric means were: group 1: 27.44 kU l⁻¹; group 2: 137.77 kU l⁻¹; group 3: 487.18 kU l⁻¹, and group 4: 4754.87 kU l⁻¹. Significance (u test according to Mann & Whitney) $p < 0.0001$ between groups 1/3, 1/4 and 2/4. Between group 1 and 2 there was no significant differences. Thus, comparison of group 3 and 4 revealed high significance with $p < 0.002$.

Here, we clearly demonstrate that both healthy individuals and patients with AD show an immune response against gp200 antigen of *S. cerevisiae*. There was a slight increase in the frequency of IgE and IgG reactions in group 3 (IgE: 63%, IgG: 100%) and group 4 (IgE: 86%, IgG: 79%) contrary to healthy group 1 (IgE: 55%, IgG: 55%), and group 2 (IgE: 67%, IgG: 89%). In patients with atopic predisposition and AD the immune response to fungi is a well known phenomenon, in particular colonization of the mucous membranes with *C. albicans* may be one factor for exacerbation of the disease [19]. Savolainen et al. [23] examined the immune response (IgE, IgA, and IgG) to environmental fungi (*C. albicans*, *C. utilis*, *Cryptococcus albidus*, *Rhodotorula rubra*, *S. cerevisiae*) in patients with AD. They found a 46 kDa-band in all investigated yeasts, as well as a 13 kDa-band in four of the investigated yeasts. The strongest immune response was found for *C. albicans* and *S. cerevisiae*, which were probably directed against the mannose residues. They did not exclude that atopic patients, primarily those who are sensitized by these fungi, may develop allergic symptoms by being exposed to other

environmental yeasts because of cross-reacting IgE antibodies. Cross reactions between *S. cerevisiae* and *C. albicans* were described in several studies. Shen et al. [24] found that the 40 kDa allergen of *C. albicans* has a homology of up to 70%, both with nucleotides and amino acid sequences of alcohol dehydrogenase of *S. cerevisiae*.

Kortekangas-Savolainen et al. [13] detected a simultaneous IgE binding to a 48 kDa band of baker's yeast and the 46 kDa band of *C. albicans*. A cross reaction between *C. albicans* and *Pityrosporum ovale* was described as well [6, 12, 22]. There are also other cross reactions reported for fungi. The minor allergens, Alt a 7 (*Alternaria alternata*) and Cla h 5 (*Cladosporium herbarum*) share high amino acid sequence homology (70%) with YCP4 protein of *S. cerevisiae*, whereas enolase from Cla h 6 showed high sequence homology with enolases from *S. cerevisiae*, *Alternaria alternata*, and *C. albicans*. Mannans are responsible for the immunologic cross-reactivity of *C. albicans* with other fungal species. Enolase of *C. albicans* share common IgE epitope with the enolase of baker's yeast *S. cerevisiae*. Similarly, the sequence ho-

mology at the protein level between enolases from *S. cerevisiae* and Cla h 6 from *Cladosporium herbarum* is reported as 83% [15].

In a recent study, Lintu et al. [17] found cross-reacting IgE and IgG antibodies in AD. In the IgE response, the main cross-reacting pattern was the mannan region, although inhibition also could be achieved with crude antigens of *C. albicans*, *S. cerevisiae*, and, to some extent, *Cryptococcus albidus*. *Pityrosporum ovale* was the most potent inhibitor of IgE-binding components. However, highest IgE antibody levels were detected in AD serum pools. In contrast, *C. albicans* was found to be the most important inducer of IgG antibodies, since the IgG level against *Pityrosporum ovale* mannan in both AD serum pools was very low.

Nevertheless, *S. cerevisiae* is generally not regarded as a human pathogen. The identification of gp200 as a soluble heatstable antigen suggests that immunological sensibilization may occur in human beings through digestion of brewing and baking products. This contrasts to infectious colonisation by intact viable microbes, as it occurs in case of the yeast *C. albicans*, a well-recognised pathogen. Thus, 10–20% of healthy individuals possess circulating IgG anti-*Saccharomyces* antibodies [2]. Interestingly, Darroch et al. [4] reported that the lymphocytes of healthy individuals show increased DNA synthesis in response to the *S. cerevisiae* antigen.

Recently, Savolainen et al. [21] found that *C. albicans* mannan induced elevated interleukin 2 and interferon χ responses in AD patients. The correlation of the cytokine responses with mannan-induced IgE and proliferation responses suggest that *C. albicans* mannan induced TH1 type cytokine responses are involved in AD. In a similar manner, *Saccharomyces* glycoprotein 200 may have immunomodulatory properties which are important for the pathogenesis of AD.

In conclusion, using two different techniques – immunoblot analysis and the immunoCAP method – both specific IgE and IgG antibodies against *S. cerevisiae* gp200 were detected in AD which is not to be explained by a cross reactivity e. g. against *C. albicans* allergens. Gp200 will be a demanding subject for future research within the immunopathogenesis of AD. Further investigations with the recombinant gp200 will give information on the role of gp200 in AD.

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