

IgE, IgA and IgG antibodies and delayed skin response towards *Candida albicans* antigens in atopics with and without saprophytic growth

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Summary

Immunoblotting and RAST were used to analyse IgE, IgA and IgG responses to antigens of *Candida albicans*. These were compared with the delayed skin response and *C. albicans* carriage in 40 atopic subjects. The majority of the atopic patients showed a strong IgG and IgA antibody response towards mannan, a carbohydrate, but only occasionally to proteins. Altogether 22 of the 40 patients showed specific IgE towards *C. albicans* by immunoblotting. The IgE response was mainly towards proteins, particularly to ones with molecular weights of 29 kD and 46 kD, and only in eight out of 22 IgE-positive subjects towards mannan. The IgG and IgA responses to mannan and the total IgE response towards *C. albicans* assessed by RAST showed an association with *C. albicans* carriage, whereas the delayed skin response showed an inverse relationship. The immunological parameters characteristic of *C. albicans* carriage were found to be *C. albicans*-specific depressed delayed skin response and elevated IgE, IgA and IgG responses. This situation in the atopics presenting such parameters may favour simultaneous sensitization and exposure by colonization. The degree of sensitization may be sufficiently high to produce symptomatic allergy, such as asthma, in some individuals during occasional overgrowth of *C. albicans*, e.g. due to antibiotic therapy.

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Introduction

During the past decades, several investigators have proposed a role for *Candida albicans* in some cases of atopic disease, particularly in asthma and rhinitis [1–3]. Although bronchial hyperreactivity to *C. albicans* extracts has been induced in some studies, direct evidence of *Candida* asthma is lacking. This is due to the use of inadequately characterized *C. albicans* extract preparation. *Candida albicans*, as well *Trichophyton*, is interesting as a probable causative agent of asthma, as allergic exposure is caused by saprophytic growth in the body [4].

Higher frequency of precipitating anti-*C. albicans* antibodies in asthma and in atopics compared to normal individuals has been shown [5]. The assessment of the allergic immune response is, however, a difficult task as

antibodies [6] and cell-mediated skin responses [7] towards *C. albicans* are common in the normal population. In a previous study, *C. albicans* colonization was found more frequently with positive immediate reaction to *C. albicans*, whereas the delayed reaction showed a negative association to *C. albicans* carriage [8].

This study was undertaken to analyse the IgE, IgA and IgG antibody responses to antigens of *C. albicans* with immunoblotting, to compare the various isotype responses to individual allergens with delayed skin response, and to find immunological parameters characteristic of *C. albicans* carriage in the nasopharynx and the gut.

Materials and methods

Subjects

Altogether 67 atopic patients were recruited into this study. These subjects were diagnosed as having asthma,

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rhinitis or atopic eczema at the University Central Hospital of Turku. The subjects were randomly selected with no manifest candidiasis or any signs of *C. albicans* growth on mucous membranes. The patients were invited to visit the departments of Dermatology and Pediatrics, University of Turku, for monthly physical examinations and serum sample collections for 8 months. In addition, bi-monthly skin tests as well as nasopharyngeal and anal swabs for *C. albicans* cultures were taken. Due to irregular attendance, 27 were excluded and 40 subjects were included in the study. Of these, 19 were males and 21 females, their mean age was 22.2 yr (s.d. = 11.2 yr) with a range of 6–45 yr. Twelve subjects were under 15 years of age. The subjects had clinically diagnosed asthma (11/40), allergic rhinitis (22/40) and atopic eczema (26/40), mainly in combinations. Thirty-five of the subjects had a previously observed immediate skin-prick test sensitivity to *C. albicans*, whereas five were *C. albicans*-negative.

Subject investigations

Intradermal skin tests were performed four times during the period with 0.1 ml of commercial Candidin (Dome/Hollister-Stier, Slough, U.K.). The diameter of the induration was measured in millimetres after 48 hr. Indurations larger than 3 mm in diameter were regarded as positive. Due to practical reasons, the data from delayed skin responses were only obtained from 36 of the 40 subjects.

Nasopharyngeal and anal cultures were taken four times during the observation period. Sera were obtained from all 40 subjects during each visit.

Immunoblotting and *C. albicans*-RASTs (Pharmacia, Uppsala, Sweden) were performed with the first and last serum samples.

Immunoblotting

The cytoplasmic *C. albicans* antigen was prepared from the lyophilized yeast cells of *C. albicans* (Strain 72024, Mycological Laboratory of the Department of Dermatology, University of Turku) as previously described [8]. The cytoplasmic *C. albicans* antigen and low molecular weight markers (Pharmacia) were separated with SDS-PAGE in a 5–17.5% gradient gel according to Laemmli [9] and transferred to nitrocellulose sheets (0.22 µm, Millipore, Bedford, U.S.A.) in Transphor apparatus (LKB, Bromma, Sweden) according to Towbin *et al.* [10].

After incubation with a subject's serum, the binding of the IgA and IgG antibodies bound to *C. albicans* antigens on nitrocellulose was detected with horseradish peroxidase (HRP)-conjugated anti-IgA and anti-IgG antibodies (DAKO Immunoglobulins, Glostrup, Denmark) as described previously [11].

The binding of the specific IgE to allergens on nitrocellulose was detected with β -galactosidase-labelled anti-IgE antibodies (Phadezym-RAST, Pharmacia) according to Bengtson *et al.* [12] as described earlier [13].

A densitometer and supplied software (Bio-Rad, Richmond, CA, U.S.A.) were used to quantify and compare the individual antibody responses to *C. albicans* antigens. The density of the scanned bands was expressed as optical density (OD) and the band width in millimetres. The integrated areas of the densitogram peaks were thus expressed as ODmm (optic density $\times$ millimetres) and values 0.1 ODmm above background were regarded as positive.

Statistics

Fischer's exact tests, Mann-Whitney *U*-tests and Spearman's rank-order correlations were used for the statistical analyses.

Results

The results from the immunoblotting experiments are presented in Figs 1 and 2. Figure 1 shows the results from the IgE detection with the *C. albicans*-RAST values obtained (Phadebas-RAST units per litre (PRU/l) of the same subject's serum sample. Twenty-two subjects of the total 40 were positive in immunoblotting, and the bands that bound IgE from the sera of these patients are summarized in Table 1. The three most important allergens were a 29 kD protein band (64%), a 46 kD protein band (41%), and the mannan polysaccharide (36%), presenting a typical diffuse high molecular weight stain [11,13] best seen in strips with PRU values of 3.47, 1.02 and 0.53.

Figure 2 presents some data from the IgG and IgA detections. The predominant antigen in IgG and IgA classes, in contrast to IgE, is mannan, with the typical diffuse high molecular weight stain [11,13]. IgA response towards mannan was seen in 72.5% and IgG-response in 82.5% of the subjects. The highest OD of the mannan stain was measured from the densitogram of each strip. Randomly selected examples in Fig. 2 represent different mannan densities and are shown with the OD values obtained.

Examples of the stained protein bands can also be seen in these strips. Occasional protein bands were seen in 21 subjects, and their molecular weights varied. The most common protein band in IgA detections was a 26 kD band found in three subjects and a 21 kD band in IgG detections in seven subjects. With a few exceptions, mannan stain was also visible with protein bands. No association with *C. albicans* carriage or delayed reaction was seen with IgA or IgG antibodies to the protein bands.

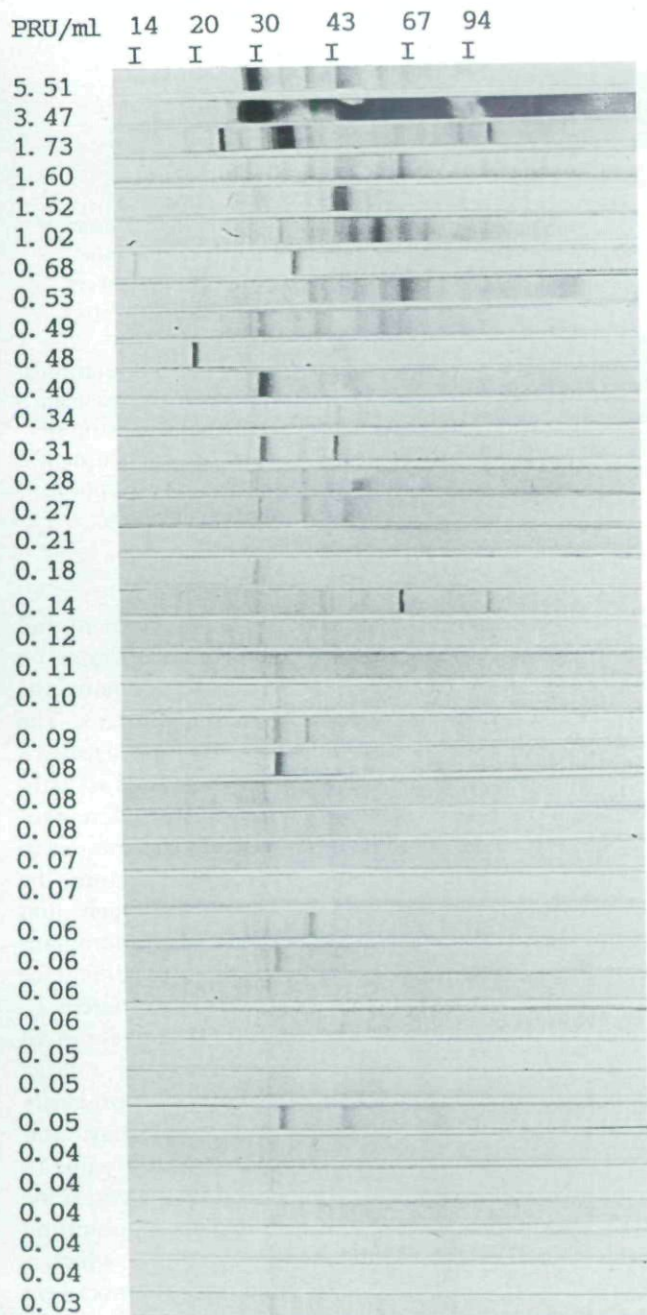


Fig. 1. IgE immunoblotting after SDS-PAGE with 40 subjects' sera. *Candida albicans* RASTs (Pharmacia) were performed with the same sera and the strips are presented in order of the PRU/l values. Reactivity of the sera in RAST decreases downward. Molecular weight markers (kD) are shown above the first strip.

Candida albicans was found more often in the nasopharyngeal than in the anal cultures. Nasopharyngeal *C. albicans* growth was found in 48%, but anal growth only in 33% of the subjects. Usually, when anal growth was seen, nasopharyngeal growth was also seen. *Candida albicans* was the most common isolate in the cultures (70.2% of all isolates). Other isolates included *Saccharomyces cerevisiae* (11.7%), *Pityrosporum ovale* (4.0%), *Rhodotorula rubra* (4.9%), *Geotrichum candidum* (4.4%) and *Candida parapsilosis* (2%). Some anterior nasal cultures were also taken and no *C. albicans* growth was found.

Only minor changes were seen in the parameters studied during the observation period of 8 months. These changes showed no association with each other, except for the reversed direction of change of delayed and humoral responses in four subjects. The changes in Candidin skin reactions were in both directions. In one subject the Candidin reaction turned to negative during the study and in another an originally negative reaction was converted to positive. 'Desensitization' and sensitization due to repeated skin testing is possible in these two subjects. The IgE response to individual allergens in the IgE immunoblotting was not frequent enough to allow comparison with other factors. The RAST values were thus taken as representative of the IgE response. The major responses of the IgG and IgA antibodies were towards mannan and only occasionally to protein antigens. The mannan OD values obtained were thus the best parameters of the IgA and IgG response. The comparison of these values with the Candidin reaction and nasopharyngeal carriage is presented in Fig. 3.

Nasopharyngeal *C. albicans* growth was more frequently found in atopics who had a negative (induration diameter less than 3 mm) cell-mediated Candidin skin reaction ($P < 0.05$, Fischer's exact test) (see Fig. 3). In contrast, the anti-*C. albicans* IgE, IgA and IgG responses were more often seen in those subjects harbouring *C. albicans* in the nasopharynx, as compared to atopics free of nasopharyngeal *C. albicans* growth (IgE: $P < 0.02$, $r = 0.34$; IgA: $P < 0.001$, $r = 0.58$; IgG: $P < 0.001$, $r = 0.65$, Spearman's rank-order correlation). The elevated anti-*C. albicans* IgA ($P < 0.05$) and IgG ($P < 0.02$) responses were significantly more frequent among the Candidin-negative atopics than among the Candidin-positive atopics (Mann-Whitney *U*-test). The same phenomenon was also observed with IgE-responses, but only with marginal significance ($P = 0.057$). Sex and age of the subjects did not show any association with the parameters studied.

Discussion

The *C. albicans*-specific IgE response was assessed by RAST and immunoblotting followed by densitometry.

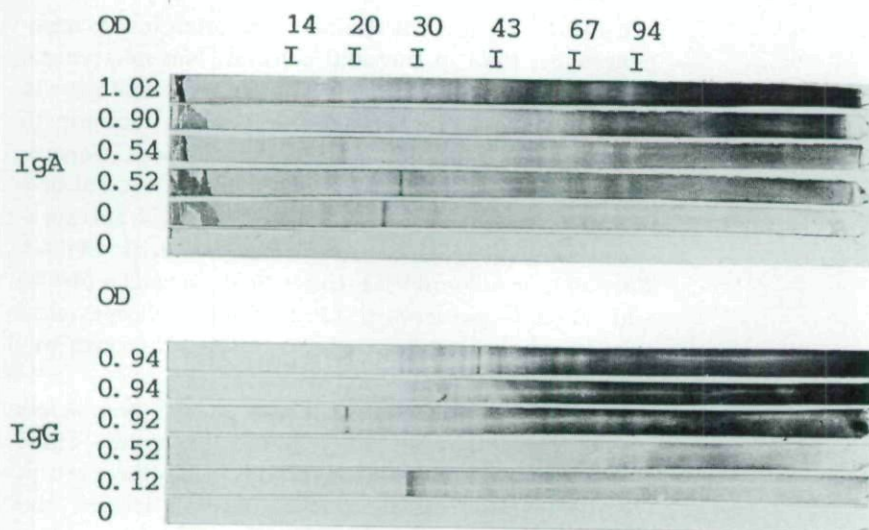


Fig. 2. Randomly selected examples of strips with IgA and IgG immunoblotting. Molecular weight markers (kD) are shown above the first strip. The diffuse staining in the high molecular weight regions of the strips is due to IgA or IgG binding to mannan. The mannan stain varies from strip to strip. The density of the mannan stain was measured and the highest optical density (OD) obtained presented.

Table 1. The IgE-binding bands of the immunoblots of Fig. 1 classified according to occurrence and intensity of the positive responses

Allergen	ODmm				Positive (>0.1)	Positive (%)
	0	<0.5	<1	>1		
Bd29K	25	5	6	3	14	64
Bd46K	28	5	2	2	9	41
BdMan*	32	3*	4*	1*	8*	36
Bd34K	33	5	—	1	6	27
Bd50K	34	4	1	1	6	27
Bd39K	34	4	—	—	4	18
Bd63K	36	1	2	1	4	18
Bd35K	36	2	1	—	3	14
Bd43K	36	3	—	—	3	14
Bd57K	38	—	1	1	2	9
Bd150K	38	2	—	—	2	9
Bd135K	38	1	—	—	1	5
Bd14K	38	1	—	—	1	5
Bd125K	39	—	1	—	1	5
Bd60K	39	—	1	—	1	5
Bd25K	39	—	1	—	1	5
Bd21K	39	—	1	—	1	5
Bd110K	39	1	—	—	1	5
Bd95K	39	1	—	—	1	5
Bd44K	39	1	—	—	1	5
Bd41K	39	1	—	—	1	5
Bd27K	39	1	—	—	1	5
Bd92K	39	—	—	—	0	—

*The corresponding limits for BdMan were taken fourfold compared to protein bands as the mannan band width is fourfold that of the proteins.

The use of the densitometer allowed measurement and comparison of the IgE response to individual allergens by integration of the obtained IgE peaks, and eliminated the effect of the micro-heterogeneity of the proteins. The analysis of our adolescent/adult atopic subjects revealed a different panel of IgE-binding bands compared to asthmatic children, 77% of whom showed a very dominant response to a 46 kD protein and only minor responses to mannan and a 29 kD protein [11]. In the present study, the 29 kD protein appeared to be a major allergen, and mannan and the 46 kD protein the most dominant intermediate allergens. This may be due to the difference in mean age between these two groups, to different *C. albicans* antigens used, or merely to two relatively small subject samples. This emphasizes the need for a large number of subjects in allergen classification, most easily obtained by immunoblotting. Immunoblotting and RAST mainly give the same results, but possibly due to qualitative or quantitative antigenic differences some RAST-negative sera give positive IgE-immunoblotting results and vice versa. Unlike the IgE-response, which is mainly towards proteins, the IgG and IgA responses were directed towards mannan with only a few protein bands. As a crude antigen preparation was used, this may be a matter of sensitivity, since a greater variety of bands was obtained with affinity-purified protein preparations [11].

The anti-*C. albicans* IgE, IgA and IgG responses had significant association with the nasopharyngeal *C. albicans* growth and a reversed association with the cell-mediated Candidin skin response. This was in agreement with the previous observations on the *C. albicans*-specific immune responses, describing inverse relationships between the delayed skin response, on the one hand, and the immediate skin reactivity [8] and IgA and IgG

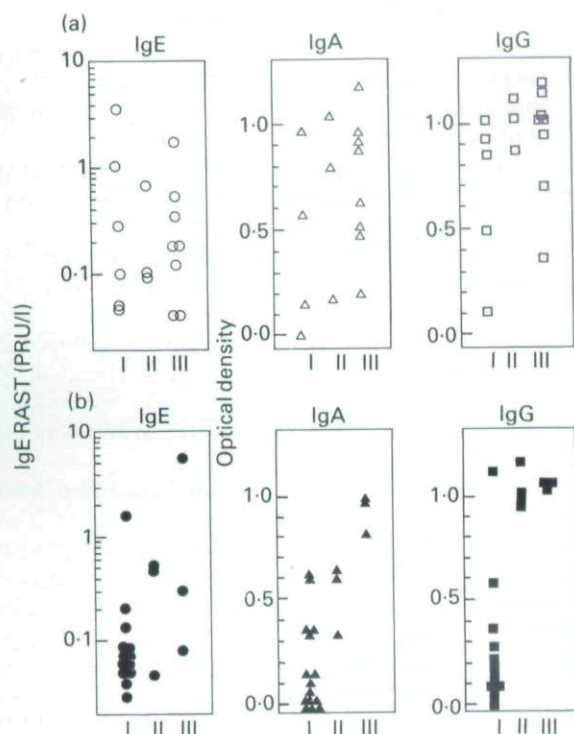


Fig. 3. The relationships between the delayed skin response to *C. albicans*, *C. albicans*-specific IgE (○, ●), IgA (△, ▲) and IgG (□, ■) and degree of nasopharyngeal growth (I: no growth, II: +/+ and III: +++). IgE measured by RAST, values stated as PRU/l; IgA and IgG determined by density of anti-mannan response in immunoblotting. Negative Candidin reactions, < 3 mm (a), and positive Candidin reactions, > 3 mm (b).

responses, on the other [14]. Typical of the atopic immune response to *C. albicans* are a strong antibody response [5] and diminished delayed skin reaction [15,16]. In this study, the *C. albicans* carriage was predominantly found in atopics who presented such a response. The elevated IgE-values against *C. albicans* detected in these subjects suggest that they are susceptible to simultaneous exposure and sensitization by *C. albicans*.

It should be investigated which one of these related factors is primary. In atopic disease the cell-mediated immune response is depressed [15,16], and a T-cell defect is believed to lie behind the abnormal IgE-response [17]. We know that in defence against *C. albicans* the cell-mediated immune system plays the major role [18], and any defect here could thus primarily cause the saprophytic *C. albicans* growth and induce specific IgE production. The presence of *C. albicans* would then stimulate the antibody responses in an effort to eliminate the yeast. This resembles the situation in leprosy [19] where the humoral immunity is stimulated in order to substitute for the defective cell-mediated immunity. The fact that the IgA

and IgG responses correlate with the estimated degree of nasopharyngeal growth lends further support to this hypothesis. The most likely explanation for the observed phenomena is, therefore, the general disturbance of the atopic immune response with depressed cell-mediated immunity. As a result we find saprophytic *C. albicans* growth. Indeed, the same may well be true if commensal skin microbes, such as *Pityrosporum* or *Trichophyton*, were studied.

Although the saprophytic growth is most certainly a consequence of the imbalance between the cell-mediated and humoral immunity, there are interesting reports indicating that *C. albicans* itself could also be an effector in making the host favour its growth. *Candida albicans* mannan has immunomodulatory effects on T cells [20] and on the co-operation between B cells and T cells [21]. Thus the commensal *C. albicans* could be capable of modulating both the cell-mediated and humoral immune responses towards itself, which may partly explain its commensalism. In a murine model, mannan given i.v. significantly suppressed the delayed hypersensitivity reaction to mannan and *C. albicans* glycoprotein [20]. The primarily depressed cell-mediated response in colonized atopics could therefore, by the immunomodulatory effects of *C. albicans*, lead to a vicious circle of impaired cell-mediated response and saprophytic growth.

What seems possible is that in atopics the suppressed cell-mediated immune response towards *C. albicans* leads to a predominant saprophytic carriage and provokes an IgE response. No correlation between the saprophytic *C. albicans* growth and allergy symptoms was found in the previous study [8]. This existing sensitization due to saprophytic growth may, however, underlie allergic symptoms developing during occasional overgrowth, for example due to antibiotic therapy in some individuals.

Our results imply that the atopic immune response may favour simultaneous sensitization and exposure by *C. albicans*. This supports the suggestions, raised by clinical findings, that *C. albicans* may indeed be an important agent in some cases of asthma and allergy.

Acknowledgments

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