

IgE-, IgA- and IgG-antibody responses to carbohydrate and protein antigens of *Candida albicans* in asthmatic children

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Analysis of IgE, IgA and IgG antibodies directed against *Candida albicans* antigens in 28 asthmatic children was performed with immunoblotting after SDS-PAGE. Analysis with the purified cytoplasmic protein fraction revealed a major protein allergen with an MW of 46 kD. In addition to the major allergen, 15 other antigenic bands with molecular weights between 16 and 135 kD bound IgE. Ten of 13 anti-*C. albicans* IgE-positive children had IgE towards the 46 kD major allergen. None of the subjects in the study group or in the non-atopic controls had IgA or IgG antibodies towards this protein. Analysis of the crude surface extract showed that mannan, a carbohydrate, was an intermediate allergen contrary to being the major antigen in IgA and IgG antibody responses.

Key words: allergen; *Candida albicans*; IgE, IgA, IgG; immunoblotting; mannans.

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CLINICAL ASPECTS

Immunoblotting analysis showed presence of IgE-antibodies to several allergens in asthmatic children. The possible role of *C. albicans* in symptomatic allergy has to be considered. The large number of allergens may offer a considerable task in the quality control of *C. albicans* extracts.

The possible role of *Candida albicans* as an allergen is still much disputed. Though evidence on *Candida* allergy has been shown (2, 7, 8, 9), knowledge of the individual allergens is limited. In studies by Longbottom et al. (13) and Edge & Pepys (5) it was shown that a carbohydrate fraction (mannan) and the cytoplasmic protein fraction contained allergens. Based on these studies, it seems that the protein fraction contains the most important allergenic determinants, and mannan contains the specific binding sites for IgG₄ (13). Mannan is known to be the most immunogenic antigen of *C. albicans*, to which most individuals have antibodies (4). Higher levels of antibodies in different immunoglobulin classes to *C. albicans* have shown slight correla-

tions to atopic diseases (6). Our previous studies focused on allergenicity of lectin purified mannan, mannoproteins and different fractions of the yeast cell. They confirmed the allergenicity of mannan and also revealed several distinct IgE binding protein bands of *C. albicans* (15, 17). The purpose of this study was to analyse IgE responses to individual allergens and compare the responses to those in IgA and IgG classes.

MATERIAL AND METHODS

Preparation of Candida albicans antigens

Yeast cell cultures. *Candida albicans* (CBS 5982) serotype A (Hasenclever), was grown on agar

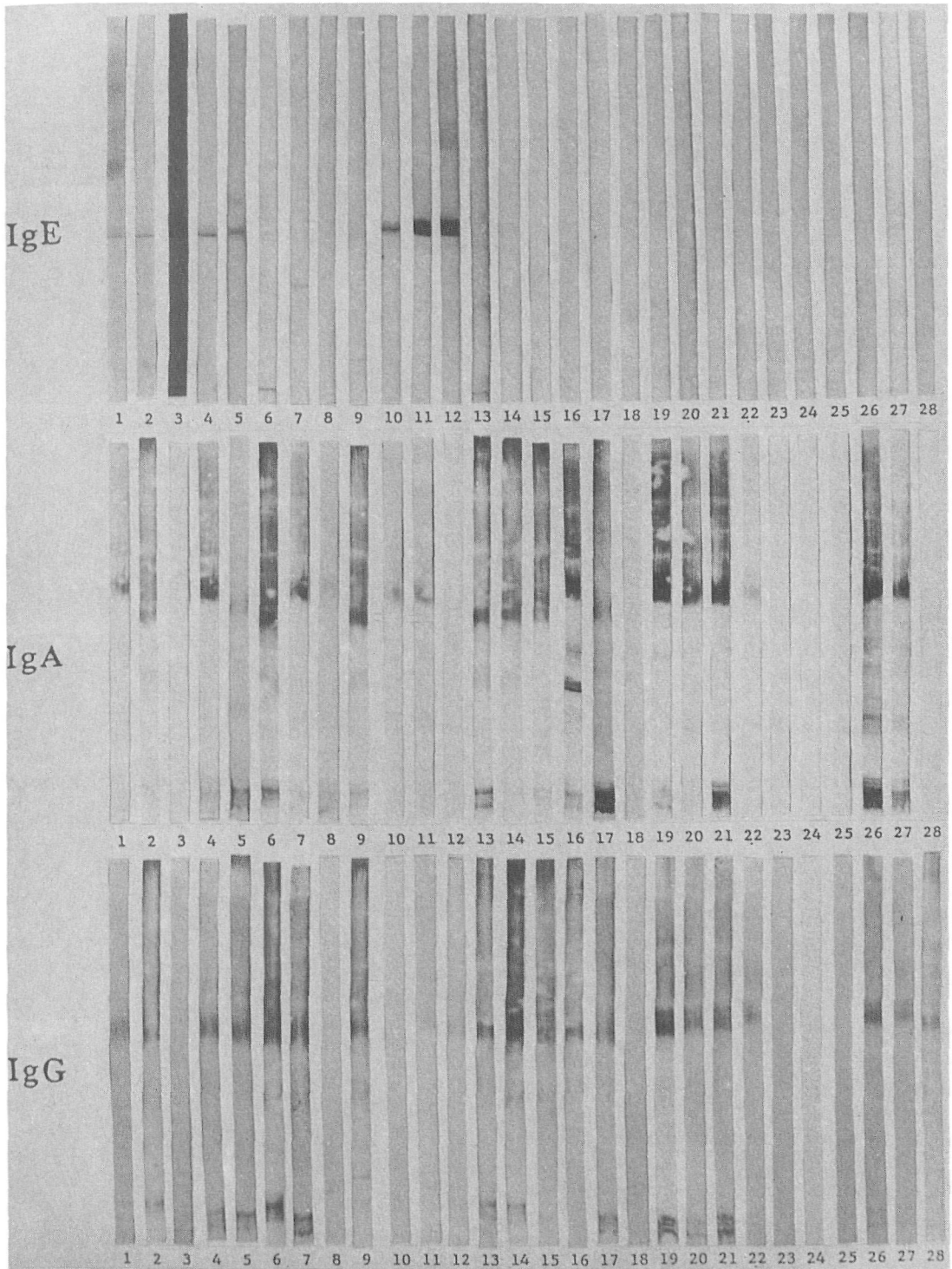


Fig. 1. Immunoblotting of the mannan-containing surface antigen. The antigen was run in 5–17.5% SDS-PAGE gel and transferred on to nitrocellulose. Nitrocellulose strips were incubated with individual patient sera and direct antibodies to immunoglobulins of different classes. The numbers refer to patients in Table 1.

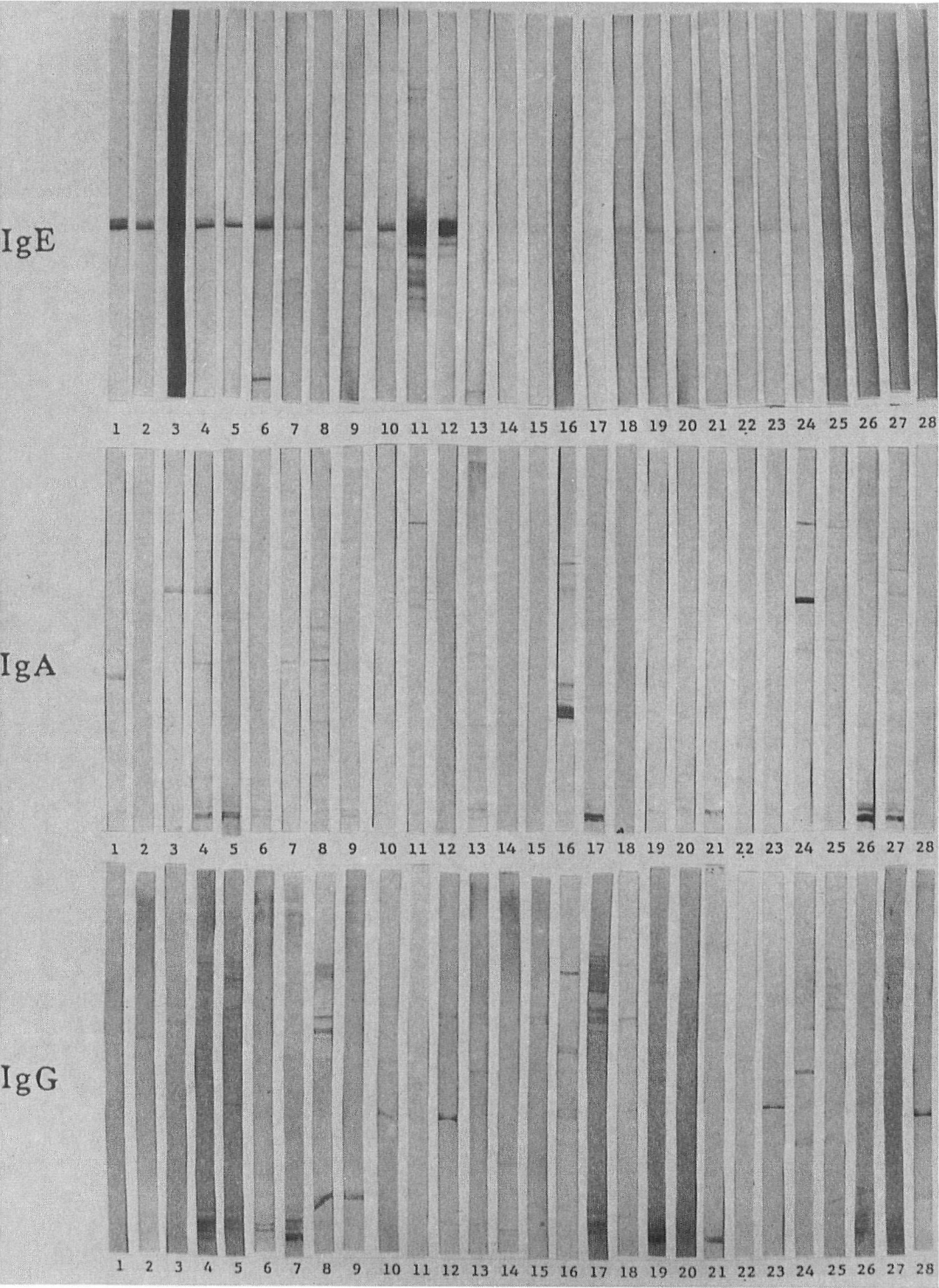


Fig. 2. Immunoblotting of the purified mannan-free protein antigen performed as in Fig. 1.

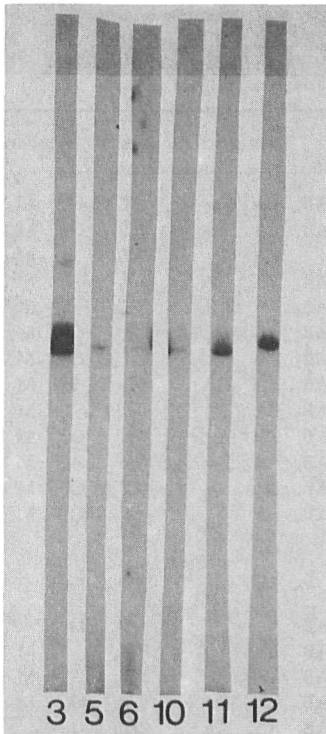


Fig. 3. Selected strips performed with ^{125}I -labelled anti-IgE and exposure on X-ray film. Case numbers are as in Table 1 and Figs. 1 and 2.

plates on a synthetic medium as previously described (15).

Surface extract. The washed yeast cells were suspended in 0.125M NH_4HCO_3 (Merck) + 0.015M Na_3N (Merck 6688) to a 20% (w/v, wet weight) suspension. The suspension was shaken for 24 h in +4°C without washing the cells, thus saving all possible surface determinants. After shaking, the suspension was centrifuged at +4°C with 30 000 g for 30 min, and the supernatant was filtered with a 0.2 μm filter and thereafter concentrated with Amicon ultrafiltration with excess of saline. The final dialysate was lyophilised and designated the surface extract, S. This material was used in immunoblotting studies as representing the surface determinants, mainly carbohydrates (17).

Crude cytoplasmic extract (CC) was prepared as previously described (15) and used in skin prick

testing as representing both carbohydrates and proteins (17). Skin prick test extract was prepared by diluting 8.3 mg (dw) lyophilised extract CC into 1 ml of NSA-diluent (Pharmacia, Uppsala, Sweden) corresponding to a 10% (w/v) of original yeast material. Before use, tests for irritability and sterility according to European Pharmacopoea were performed.

Purified cytoplasmic extract. The crude cytoplasmic extract was purified with ConA-Sepharose affinity chromatography as described earlier (15) to obtain a purified protein extract (PP), which was used as antigen in immunoblotting experiments as representing the cytoplasmic determinants, proteins (17).

Patient investigations

Patients for the study were selected from the Children's Asthma Register, Turku University Central Hospital (10), using the following criteria: a previous *C. albicans* SPT result ++ or more, and/or previous *C. albicans* RAST score 1 or more, and age under 15 years. Permission for patient investigations was obtained from the ethics committee of the University Central Hospital of Turku. Altogether 31 children attended the study. Three patients refused either skin prick test or blood sample, and were excluded leaving 28 children for the study.

Prick tests were done and read according to Nordic recommendations (1) except that the results were calculated as indexes as previously described (11). Briefly, the medium diameter of the wheal was subtracted from the medium diameter of the control wheal and divided by the medium diameter of the histamine wheal.

Serum samples were taken for *C. albicans* RAST (Pharmacia diagnostics, Uppsala, Sweden) and for immunoblotting. As controls, nine non-atopic sera were analysed for IgE-, IgA- and IgG-antibodies against *C. albicans* with immunoblotting.

SDS-PAGE and immunoblotting.

SDS-PAGE and transfer on to nitrocellulose. *C. albicans* extracts (S and PP) and molecular weight

Table 1

Data concerning patients in the study. Age and asthma onset age are counted from the nearest birthday. SPT results are presented as indexes. RAST values are given in PRU/ml

		Age	Asthma onset age	SPT (index)	RAST (PRU)	Diagnoses	Allergens	
1	M	11	5	0.8	1.41	AB, EA	T, G, M, A	
2	F	9	4	0.8	0.51	AB, EA, RA	T, G, M, A	
3	F	5	3	1.6	0.67	EA	T, M, A	F
4	F	8	2	0.7	0.38	AB, EA, RA	M, A	
5	M	11	2	0.4	0.07	AB, RA	T, M, A	
6	M	10	3	0.8	0.08	AB,	T, G, M, A	
7	M	7	1	0	0.09	AB, EA	T, G, M, A	
8	M	9	2	1.3	0.05	AB, EA, RA	M, A	
9	M	7	2	1.0	0.49	AB, EA	M, A	
10	M	10	1	1.5	0.24	AB, EA	G, M, A	
11	M	8	2	1.3	2.00	AB, EA	M, A, A	F
12	F	10	3	1.6	1.98	AB, EA	T, G, M, A, A	F
13	M	11	5	0.4	0.61	AB, EA	T, G, M, A	
		9.5 (2.4)	2.6 (1.4)	0.94 (0.50)	0.66 (0.69)			
14	F	8	1	0	0.07	AB	T, G	
15	M	8	4	0	0.03	AB	T, M	
16	M	9	4	1.0	0.17	AB	T, G, M	
17	M	15	1	1.3	0.11	AB, EA, RA	T, G, M, A	
18	M	10	3	0	0.03	AB, EA, RA	T, G	
19	M	11	5	0	0.04	AB, EA	T, G, M, A	
20	M	9	8	0	0.10	AB, EA		
21	M	10	6	0	0.06	AB, EA, RA	G, A	
22	M	11	8	0	0.06	AB	T, G, A	
23	M	14	3	0	0.17	AB, EA	T, G, A	
24	M	10	4	0	0.20	AB, EA	T, G, M, A	
25	M	13	2	0	0.07	AB, EA,	T, G, A	
26	M	14	2	1.0	0.08	AB	T, G, A	
27	M	9	7	0	0.10	AB, EA	T, G, A, A	F
28	F	13	9	0.9	0.13	AB, EA, RA	T, G, A	
		10.9 (2.3)	4.6 (2.9)	0.28 (0.49)	0.09 (0.05)			

Diagnoses are AB = bronchial asthma, EA = atopic eczema and RA = allergic rhinitis. Column Allergens gives allergens to which the patient according to skin tests and/or RAST is allergic: T = trees, G = grasses, M = moulds, A = animals and F = foods. Patients are presented in two groups: numbers 1–13 are IgE-positive in immunoblotting and numbers 14–28 IgE negative.

markers (LMW Calibration Kit, Pharmacia Fine Chemicals, Uppsala Sweden) were separated with SDS-PAGE in 5–17.5 % gradient gel according to Laemmli (12) and transferred to nitrocellulose sheets (Millipore 0.22µm, Millipore Co, Bedford, MA., USA) in Transphor apparatus (LKB, Bromma, Sweden) according to Towbin et al. (18).

IgA- and IgG-class antibody detection. The whole nitrocellulose sheets were incubated in 10 %

horse serum in 0.05M phosphate-buffered saline containing 0.5 % Tween 20 (HS-PBS-T) for 1 h at room temperature to saturate unoccupied binding sites. After washing three times with PBS, the sheets were cut into 5 mm broad strips and incubated over night at + 4°C with patient sera diluted 1:50 in 10 % HS-PBS-T for both IgA- and IgG-detection. After washing, the strips were incubated for 4 h at room temperature on a shaker with horseradish peroxidase (HRP) -conjugated anti-IgA

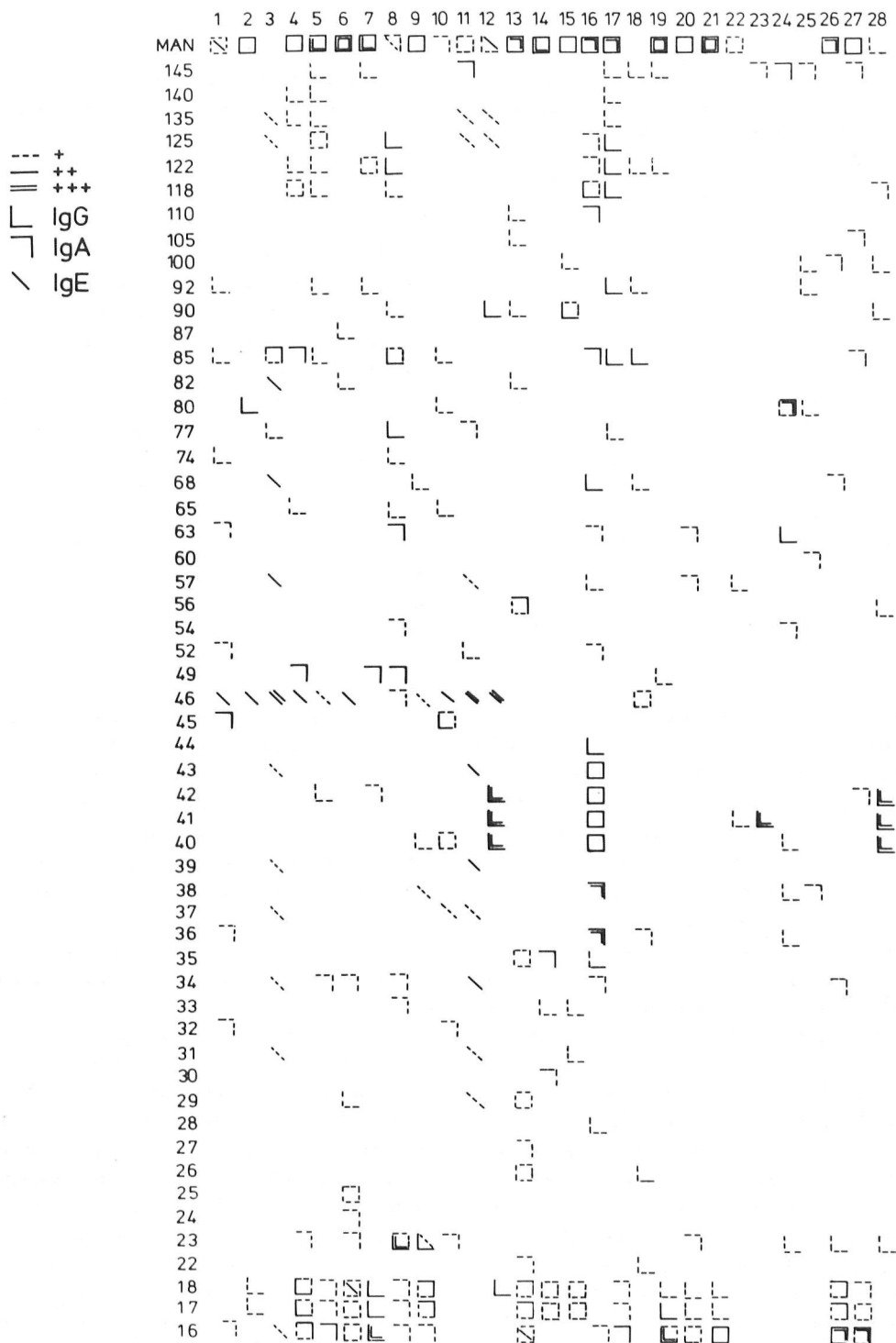


Fig. 4. Idiotypogram of the immunoblotting results presented in Figs. 1 and 2. All isotypes are included and indicated with different symbols. The outline of the symbols refers to strength of the staining obtained in immunoblotting with + for weak stain, ++ for intermediate stain and +++ for strong stain. Patient numbers are 1-28.

Table 2

Allergens of *Candida albicans* classified as major, intermediate and minor allergens based on the immunoblotting results. (MW = molecular weight of the allergen n = number of patients with specific IgE towards the allergen, % = percentage of all *C. albicans* IgE-positive patients ($N = 13$))

MW	n	%	classification
man	3	23	IM
135	3	23	IM
125	3	23	IM
82	1	8	minor
68	1	8	minor
57	2	15	IM
46	10	77	major
43	2	15	IM
39	2	15	IM
38	1	8	minor
37	3	23	IM
34	3	23	IM
31	2	15	IM
29	1	8	minor
23	1	8	minor
18	1	8	minor
16	2	15	IM

(DAKO immunoglobulins, Denmark) antibodies diluted 1:750 and anti-IgG antibodies diluted 1:1000 in HS-PBS-T. After washing, the HRP-labelled bands were detected as previously described (17). For each run, one strip of both antigens was incubated with anti-*C. albicans* antibodies (DAKO immunoglobulins, Denmark) diluted 1:50 and thereafter with HRP-conjugated anti-rabbit swine antibodies (DAKO immunoglobulins, Denmark) diluted 1:1000. These strips were used as standards for both antigen migration and peroxidase staining

IgE class antibody detection. The specific IgE binding to allergens on nitrocellulose was detected according to Bengtson et al. (3) as previously described (15). The dilution of individual patient sera as well as the dilution of the beta-galactosidase-labelled anti-IgE antibodies (Phadezym-RAST, Pharmacia Diagnostics, Uppsala, Sweden) was 1:4. Also ^{125}I -labelled anti-IgE antibodies (Phadebas-RAST, Pharmacia Diagnostics, Uppsala, Sweden) were used diluted to 20 000 cpm/ml in PBS-Tween. The galactosidase-labelled bands were detected as previously

described (15). The ^{125}I -labelled bands were detected with an X-ray film (Trimax) in a cassette with intensifying screens (Trimax) after storage for 1 week in -70°C prior to developing.

RESULTS

Figs. 1 and 2 show the results of immunoblotting with enzyme-label of all individual sera using two *C. albicans* allergen preparations. Fig. 3 presents some immunoblotting experiments performed with the isotope label. The data from patients with a corresponding number are presented in Table 1. Patients with numbers 1 to 13 have anti-*C. albicans* IgE according to immunoblotting, whereas patients with numbers 14–28 have not. The difference in RAST and SPT between these groups is evident, a certain trend is obvious with asthma onset age, whereas age, sex, diagnoses or allergy to other common allergens show practically no difference.

Fig. 1 presents the immunoblotting results using the surface antigen. Only three patients have IgE antibodies against mannan, which is best seen in strip No. 1 as a diffuse staining in the high molecular weight area. A 46 kD major protein allergen is prominent. The dominating staining due to anti-mannan IgA and IgG in the high molecular weight regions of the nitrocellulose strips can be seen in all but a few patients.

Fig. 2 shows the results with the purified protein antigen of *C. albicans*. The dominating major allergen with an MW of 46 kD in the IgE strips is seen even more clearly than in Fig. 1. All but three of the IgE-positive subjects had a strong response towards the 46 kD protein. Four patients had IgE towards other proteins, and two to a variety of them still presenting the strongest response to the 46 kD major allergen. In contrast to the IgE responses, the IgA and IgG responses to these purified proteins are polyspecific and faint, with practically no response to the 46 kD major allergen.

Though some of the strongest responses to the 46 kD allergen are almost overwhelming in the isotope-labelled strips (Fig. 3), most of the proteins with weak binding and mannan were

still absent after 1 week's exposure. Compared with the enzyme label, the isotope label seems to give too strong a contrast between strong and weak IgE-responses. The faint bands at 94 and 46 kD sites in the negative IgE strips are absent in isotope-labelled strips but present in strips laid directly in substrate with no preceding incubations with either serum or conjugate (unpublished results). Most certainly, these bands are due to the beta-galactosidase activity in *C. albicans* itself, and can be distinguished from the specific IgE binding by use of isotope-labelled strips or native substrate strips. Both of the strips of patient No. 3 (Figs. 1 and 2) turned dark red rapidly in substrate and presented a number of protein bands which could be analysed but not reproduced in a photograph from the dark strip. Some of these are seen in isotope-labelled strips. This rapid staining was apparently due to the high total IgE in this patient. She had S-IgE levels reaching 30 000 kU/l.

Fig. 4 presents an idiotypogram of the IgE, IgA and IgG responses to all detected *C. albicans* antigens. Common antigens for IgE and IgA and/or IgG are seldom seen. Also common cytoplasmic protein antigens to IgA and IgG only occur occasionally. Remarkable is the frequent co-existence of antibodies to both mannan and 16 to 18 kD proteins. Fig. 5 shows a similar kind of diagram from the control material. The absence of IgE and presence of IgG and IgA anti-mannan antibodies are evident. Antibodies

occur to a lesser number of antigens than in the asthmatic patients.

Table 2 summarizes the identified allergens of *C. albicans* as well as the degree of specific IgE binding. One major, 10 intermediate and six minor allergens were identified in the investigated patient panel.

DISCUSSION

In this pediatric atopic material, the IgE response against *C. albicans* allergens proved to be extremely monospecific and was primarily directed against a major protein allergen with an MW of approximately 46 kD. Surprisingly, the IgG- and IgA- antibody populations responded poorly to this protein. This immunoreactive protein is heterogeneous, composed of one band with strong IgE binding and a second band positioned at a slightly higher molecular weight, also with IgE binding. The IgE response to the first band is extremely strong, especially when isotope-labelled anti-IgE is used. Individual variations of the IgE response are seen with the minor and intermediate allergens. It is of interest that one such (29 kD) protein, found to be only a minor allergen, becomes a dominant intermediate allergen in adult material (16).

The dominant antigen for IgG and IgA class antibodies is mannan, visualized as diffuse staining in the high molecular weight portion of S strips (15). Most individuals have precipitations towards mannan (4), and the strong response of IgA- and IgG- antibodies is also well-visualised in immunoblotting. In the earliest reports on allergens of *C. albicans*, Longbottom and co-workers showed that the immediate hypersensitivity to mannan is mediated by short-term sensitizing antibodies, as judged by the PCA-test (passive cutaneous anaphylaxis) in guinea pigs (13). In later studies, Edge & Pepys used the RAST technique to show that IgE, as well, is involved in immediate hypersensitivity to mannan (5). Our results confirm this (15). We further show that in IgE-mediated allergy to *C. albicans*, mannan is merely an intermediate allergen, whereas it is the most immunogenic antigen in IgA and IgG response.

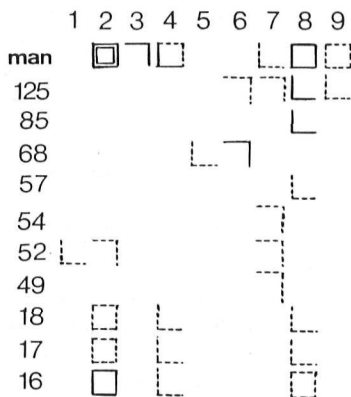


Fig. 5.

In our previous work (17), we showed that both mannan and the 46 kD protein are extracted in the first washing solutions of the intact yeast cells. Thus these two antigens are among the first determinants of saprophytic *C. albicans* an individual is exposed to. It is of interest that the IgE response, on the one hand, and the IgA and IgG responses, on the other, are divided between these two antigens. Since most allergens are proteins, and not carbohydrates, it is not surprising that IgE responses to the protein are elicited. A puzzling question remains, however, in that despite the IgA and IgG antibody responses to mannan and to random cytoplasmic determinants, there are no IgG and IgA antibody responses to the 46 kD protein. The suggestion that strong IgE binding might inhibit potential IgA and IgG responsiveness does not provide a satisfying explanation. The fact that neither atopics with negative IgE responses to the 46 kD allergen nor non-atopic controls had IgG or IgA responses to this allergen, strongly argues against the above suggestion.

The associations between the IgE, IgA, IgM, IgG and IgD levels against *C. albicans* and atopy (rhinitis and asthma) have been studied by Edge & Pepys (6). The levels of all these antibodies were higher in atopic patients than in controls. In our purely asthmatic pediatric material, there were strong IgG and IgA anti-mannan responses in almost all patients, and varying polyspecific responses to cytoplasmic proteins. Neither of these isotypic responses seems to differ from isotypic responses of non-atopics. Thus the only remaining significant connection to allergy is the specific IgE response to *C. albicans*.

Candida albicans is not an airborne allergen; the only source of exposure is, apparently, saprophytic growth in the body (14). Our previous study showed that many allergens are located on the surface of the yeast cell. Our data further indicate that IgE antibodies, as one entity, and IgG and IgA antibodies, as a different entity, recognize different determinants of *C. albicans*. Thus, the blocking of *C. albicans* allergenic epitopes by IgA or IgG antibodies seems highly unlikely. That this proposed model may be

clinically relevant is compatible with, for example, *C. albicans* exposure to mucous membranes eliciting IgE-mediated allergic reactions in sensitized atopics (2, 7, 8, 9). Further studies are needed to fully evaluate the immunoregulatory events in the humoral immune response to *C. albicans*, and its relevance to the development of symptomatic allergy.

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