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Original article

Cross-reacting IgE and IgG antibodies to *Pityrosporum ovale* mannan and other yeasts in atopic dermatitis

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Atopic dermatitis (AD) patients often demonstrate positive skin prick test results and serum IgE antibodies to a range of different yeasts. This has been thought to be due to cross-reactivity. In this study, the cross-reactivity of IgE and IgG antibodies between mannan and crude antigens of *Pityrosporum ovale*, *Candida albicans*, and *Saccharomyces cerevisiae* and crude antigens of *Cryptococcus albidus* and *Rhodotorula rubra* was examined by RAST and ELISA inhibition with two serum pools of AD patients. We found cross-reacting IgE and IgG antibodies. In the IgE response, the main cross-reacting pattern was the mannan region, although inhibition could be achieved also with crude antigens of *C. albicans*, *S. cerevisiae*, and, to some extent, *C. albidus*. *P. ovale* was the most potent inhibitor of IgE-binding components, and against it the 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 *P. ovale* mannan in both AD serum pools was very low. Cross-reacting antibodies were also seen in ELISA inhibition with both crude and mannan antigens, but since the IgG antibody level of *P. ovale* mannan in AD serum pools was low, further studies are needed to confirm the IgG results.

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Atopic dermatitis (AD) patients with immediate skin prick test (SPT) reactivity to different yeasts are exposed to yeast allergens from different sources (1). *Pityrosporum ovale* is a common saprophytic yeast of the normal human skin flora, but can act as an allergen in AD patients, especially in patients with dermatitis of the head, neck, and shoulder (HNS) region (2–7). A strong association between the

severity of AD and specific IgE antibodies to *P. ovale* has been found (8). *Candida albicans*, another common saprophytic yeast colonizing the gastrointestinal tract, has been supposed to play a role in asthma and allergic rhinitis (9, 10). Elevated IgE levels against *C. albicans* have also been found among AD patients (11). Exposure to yeasts can also take place through food containing *Saccharomyces cerevisiae*, commonly known as baker's yeast, and through airborne contacts, since several yeasts are aeroallergens; e.g., *Cryptococcus* and *Rhodotorula* (12).

Patients with allergies to fungi often demonstrate broad patterns of positive SPT results and specific serum IgE antibodies to a range of different yeasts (1, 13, 14). These results suggest extensive cross-reactions between yeast antibodies, as indicated by limited studies so far undertaken. Previously, a combination of IgE immunoblotting and RAST inhibition has revealed cross-reacting allergenic components in *P. ovale* and *C. albicans* crude extracts (15). Binding patterns suggested that the cross-reacting allergen is mannan. Similar results were obtained in a study which showed nearly complete reciprocal inhibition in the IgE ELISA (enzyme-linked immunosorbent assay) with crude extracts of *P. ovale* and *C. albicans* (16). It was also demonstrated that the cross-reactive components belonged to the pool of Con A-binding glycoproteins and/or polysaccharides (17).

So far, the cross-reactivity of yeast antibodies has not been studied with *P. ovale* mannan. Therefore, we have studied in more detail cross-reacting IgE and IgG antibodies to purified *P. ovale* mannan and the following other yeasts: *P. ovale* crude, *C. albicans* mannan and crude, *S. cerevisiae* mannan and crude, *Rhodotorula rubra* crude, and *Cryptococcus albidus* crude. RAST-inhibition and ELISA-inhibition methods were used in this study.

Material and methods

Patient sera

The serum samples were obtained from AD patients at the Department of Dermatology, University Hospital of Turku (Finland). The diagnosis of AD was based on the criteria of Hanifin & Rajka (20) and was established by a dermatologist.

Four sera with high IgE antibody level against *P. ovale* mannan were selected to form a *P. ovale* mannan-specific serum pool (pool I), according to our previous immunoblotting and *P. ovale* mannan RAST results (18). The other serum pool consisted of two sera with IgE antibodies

especially against *C. albicans*, but it also displayed a lower level of IgE antibodies against *P. ovale* (pool II). These two sera were previously studied with *C. albicans* mannan RAST and immunoblotting (19). A serum from a patient with no history of AD and negative skin prick tests was used as a control. To detect IgG antibody levels of individual AD patients, we selected eight serum samples from AD patients with previous demonstration of elevated levels of IgE antibodies to yeasts.

Antigens

All antigen extracts were prepared in the laboratories of clinical allergology and medical microbiology, University of Turku, as previously reported (1, 18, 19, 21, 22). Briefly, *P. ovale* (CBS 7854) was grown on agar plates on medium containing reduced fat cream supplementum, according to Leeming & Notman (23). The *P. ovale* crude cytoplasmic extract was prepared with disruption of the cells in an X-press (AB Biox, Gothenburg, Sweden) chamber (18, 21). *P. ovale* mannan was purified according to the Cetavlon method of Nakajima & Ballou (18, 24). *C. albicans* (CBS 5982) was grown on agar plates on synthetic medium, as previously described (21), and *C. albicans* mannan was also prepared by the Cetavlon method (19, 24). *S. cerevisiae* was obtained from Oy Alko Ab. It was a commercial product for household use (22). *S. cerevisiae* mannan was prepared by the method described by Peat et al. (19, 25). *R. rubra* crude strain 70765 and *C. albidus* crude strain 71078 were supplied and typed by the mycological laboratory of the Department of Dermatology, University of Turku, as previously reported (1). The mannan extracts were found to be free of endotoxin by E-toxate kit (Sigma).

RAST inhibition

RAST inhibition was performed with two different AD serum pools and the nonatopic control serum, according to Yman et al. (26), except that nitrocellulose disks were used as the solid phase instead of CnBr-activated paper disks. The preparation of nitrocellulose disks was carried out as previously described (27). The disks were incubated for 2 h at 37°C in antigen solutions containing 1 mg/ml for each crude antigen, 0.15 mg/ml for *P. ovale* or *S. cerevisiae* mannans, and 0.1 mg/ml for *C. albicans* mannan with phosphate-buffered saline (PBS), pH 7.4, as a diluent. A volume of 1 ml of each solution was used to coat 25 disks. In the RAST inhibition, as inhibiting fluid-phase antigens, crude and mannan extracts were used in 10-fold dilutions

from 10 mg/ml in PBS, and 25 µl of inhibiting antigen diluent and 25 µl of serum pool were mixed and incubated at 20°C for 2 h. Thereafter, the disks were added for incubation overnight at 20°C. After washing three times with PBS, ¹²⁵I-labeled anti-IgE was added for 2 h at 37°C. Radioactivity was detected by a gamma counter, and results were expressed as RAST index (sample-buffer)×100/(total activity-buffer) and as percent inhibition. PBS was used as a buffer.

Mannan ELISA

P. ovale and *C. albicans* mannan-specific IgG antibodies were measured with the ELISA technique [28]. In brief, wells of microtiter plates (Nunc, Roskilde, Denmark) were coated with 100 µl of mannan antigens (0.15 mg/ml of *P. ovale* and 0.1 mg/ml of *C. albicans* mannan) in PBS-ELISA at 4°C overnight. Blocking of microwells was carried out with 1% BSA (bovine serum albumin)-PBS for 1.5 h at 37°C. After washing, 100 µl of each serum sample (1:250 dilution in 1% BSA-PBS) or serum pool (1:125 dilution) was added and incubated for 2 h at 37°C. After incubation and washing, 75 µl/well in 1:300 dilution in 1% BSA-PBS of alkaline phosphatase-conjugated goat anti-human IgG was added to the wells for absorbance (OD₄₀₅) detection.

ELISA inhibition

ELISA inhibition was performed in the same way as the mannan ELISA already described, only it included fluid-phase inhibition of serum and inhibiting antigen. Wells of microtiter plates were coated with 1 mg/ml of crude yeast antigens (*P. ovale*, *C. albicans*, *S. cerevisiae*, *C. albidus*, and *R. rubra*), 0.15 mg/ml of *P. ovale* and *S. cerevisiae* mannans, and 0.10 mg/ml of *C. albicans* mannan. Serum pools I and II were diluted in 1/62 in 1% BSA-PBS. All yeast extracts were used as inhibiting antigens in 10-fold dilutions from 10 mg/ml.

Results

Fig. 1 shows inhibition of anti-*P. ovale* mannan IgE by fluid-phase incubation with extracts of other yeasts (Fig. 1a) and inhibition of other yeasts by fluid-phase incubation with *P. ovale* mannan extract (Fig. 1b) with serum pool I. *P. ovale* crude antigen had a strong capacity to inhibit *P. ovale* mannan (autoinhibition of *P. ovale* mannan was 100%). Other yeast extracts did not reveal any inhibition (Fig. 1a). Fig. 1b shows that *P. ovale* mannan inhibited about 60% of

IgE binding to *C. albicans* and *S. cerevisiae* mannans. A low concentration of *P. ovale* mannan extract could reach the 50% inhibition level. *C. albicans* crude and *C. albidus* crude were also inhibited by *P. ovale* mannan, but *P. ovale* could not inhibit *S. cerevisiae* crude and *R. rubra*.

Fig. 2 shows inhibition of anti-*P. ovale* mannan IgE by fluid-phase incubation with extracts of other yeasts (Fig. 2a) and inhibition of other yeasts by fluid-phase incubation with *P. ovale* mannan extract (Fig. 2b) with serum pool II. *P. ovale* mannan was inhibited by 60–70% by *C. albicans* and *S. cerevisiae* mannans, the 50% inhibition level being reached by 5–7 mg/ml of *C. albicans* and *S. cerevisiae* mannans (autoinhibition was 100%). Cross-reactivity was clearly seen between mannans. Among other inhibitor extracts, only *P. ovale* and *C. albicans* crude extracts reached the 50% inhibition level, others reaching only the 45% level with mild inhibition of *P. ovale* mannan. Fig. 2b shows that *P. ovale* mannan could inhibit *C. albicans* and *S. cerevisiae* mannans by up to 60–70%. In this case, *P. ovale* mannan, surprisingly, inhibited *C. albicans* crude antigen better than *C. albicans* mannan. *S. cerevisiae*, *R. rubra*, and *C. albidus* crude extracts did not reach the 50% inhibition level when inhibited by *P. ovale* mannan. As control, RAST inhibition with nonatopic sera showed no inhibition.

Table 1 presents IgE nitrocellulose RAST indexes for different yeast antigens in the studied serum pools. Table 2 shows IgG levels measured with inhibition ELISA. IgG antibody level against *C. albicans* mannan was highest in both pools, but in contrast to IgE results, levels of IgG antibodies against *P. ovale* mannan were low. In general, the level of IgG antibodies in both AD pools was low. In pool II, only the response to *C. albicans* was elevated; others were near background levels. Pool I had more reacting IgG antibodies, anti-*C. albicans* mannan and crude antigen being strongest. In these serum pools, *P. ovale* mainly raised IgE antibodies and *C. albicans* IgG antibodies.

Since IgG levels were low in two serum pools, we also wanted to test individual AD patient sera to learn more about the levels of IgG antibodies. We tested IgG levels against *C. albicans* and *P. ovale* mannans. In all eight AD sera tested, *C. albicans* mannan IgG-antibody levels (1.58–2.19 OD, mean background 0.08 OD) were 3–7 times higher than *P. ovale* mannan levels, which were low (0.21–0.71 OD, mean background 0.08 OD) (not shown).

Fig. 3a shows inhibition of anti-*P. ovale* mannan IgG by fluid-phase incubation in serum pool I with other yeasts, and Fig. 3b shows inhibition of other yeasts by fluid-phase incubation in serum pool I with *P. ovale* mannan. Fig. 3a shows *P. ovale* mannan inhibited by itself, *P. ovale* crude,

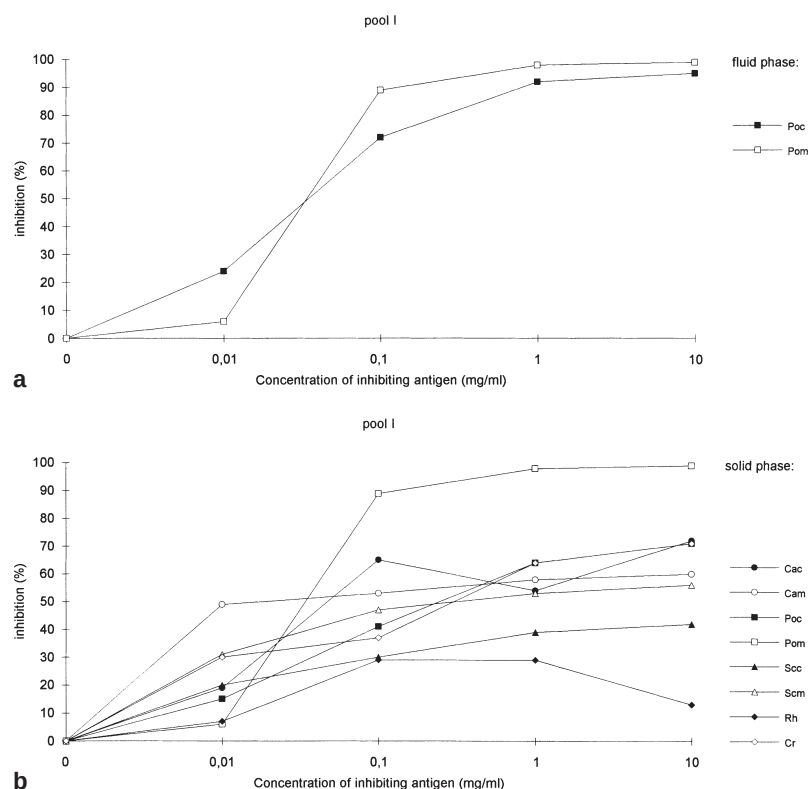


Figure 1. a) Inhibition of IgE-binding to solid-phase *P. ovale* mannan by fluid-phase incubation of other yeast extracts. □: *P. ovale* mannan (Pom), ■: *P. ovale* crude antigen (Poc), ○: *C. albicans* mannan (Cam), ●: *C. albicans* crude antigen (Cac), △: *S. cerevisiae* mannan (Scm), ▲: *S. cerevisiae* crude antigen (Scc), ◆: *R. rubra* (Rh), and ◇: *C. albidus* (Cr). Inhibition was seen only with *P. ovale* crude and mannan extracts. b) Inhibition of IgE-binding to solid-phase extracts of other yeasts by fluid-phase incubation of *P. ovale* mannan. Nitrocellulose was used as solid-phase immunosorbent matrix, and serum pool I as reacting serum.

R. rubra, *C. albidus*, and *S. cerevisiae* crude and mannan. *C. albicans* was different from the others, since its ELISA results seen in Table 2 were three times higher than *P. ovale* mannans, although it could not act as a clear inhibitor. As seen in Fig. 3b, *P. ovale* mannan inhibited all other yeasts. Cross-reacting IgG antibodies between *P. ovale* mannan and other yeasts seemed to exist. However, the low OD level of *P. ovale* mannan should be taken into consideration.

Inhibition ELISA was performed also with serum pool II, but OD levels were too low for interpretation of the inhibition results (shown in Table 2).

Discussion

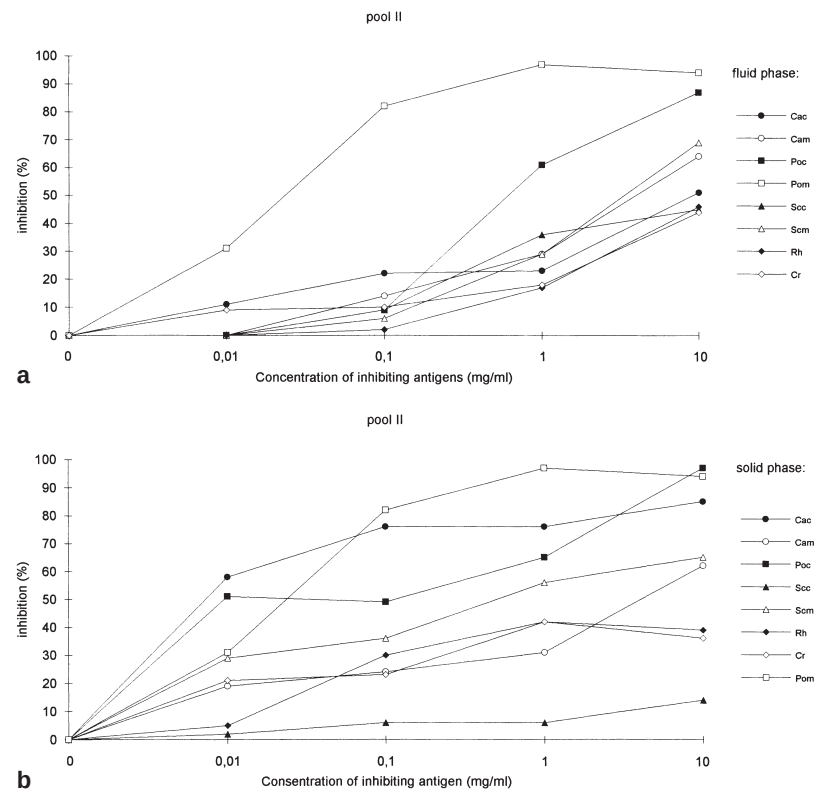
Highly significant correlation has been found between anti-*P. ovale* and anti-*C. albicans* IgE antibody levels in AD patients (6, 8). This correlation has been claimed to be due to cross-reactivity of IgE-binding components of Con A-binding glycoproteins and/or polysaccharides by Doekes & van Ieperen-van Dijk (16). In our earlier study, we found positive *P. ovale* mannan RAST in 77% of AD patients with positive commercial *P. orbiculare* RAST, suggesting that mannan is an important allergen of *P. ovale* (18). In the

present study, cross-reactivity was for the first time investigated with purified *P. ovale* mannan. We showed that there were cross-reacting antibodies between yeast mannans in both the IgE and the IgG response.

In pool I, there were a high IgE antibody level against *P. ovale* mannan and a low one against *C. albicans* and *S. cerevisiae* mannans. Pool II contained IgE antibodies against *P. ovale* mannan, but also far more antibodies against other mannans. Since the specific IgE level of *P. ovale* in serum pool I was higher than the indexes of other yeasts, strong *P. ovale* mannan could possibly eliminate inhibition when *P. ovale* mannan disks were used, since it could itself inhibit many other yeasts. Alternatively, it had common epitopes with other yeasts, but also many of its own epitopes, which could not be inhibited by other yeasts. However, findings with serum pool II showed the cross-reacting antibodies between *P. ovale* mannan and other yeast mannans.

Savolainen & Broberg (15) found earlier that *C. albicans* crude extract could partly inhibit the IgE binding to *P. ovale* crude extract in two out of five sera tested. On the basis of immunoblotting and the RAST-inhibition pattern, they concluded that cross-reacting epitopes reside mainly in the mannan of *C. albicans* and in the mannan-rich high-

Figure 2. a) Inhibition of IgE-binding to solid-phase *P. ovale* mannan by fluid-phase incubation of other yeast extracts. □: *P. ovale* mannan (Pom), ■: *P. ovale* crude antigen (Poc), ○: *C. albicans* mannan (Cam), ●: *C. albicans* crude antigen (Cac), △: *S. cerevisiae* mannan (Scm), ▲: *S. cerevisiae* crude antigen (Scs), ◆: *R. rubra* (Rh), and ◇: *C. albidus* (Cr). b) Inhibition of IgE-binding to solid-phase extracts of other yeasts by fluid-phase incubation of *P. ovale* mannan. Nitrocellulose was used as solid-phase immunosorbent matrix, and serum pool II as reacting serum.



molecular-weight diffuse band of *P. ovale* (15). The findings of Doekes & van Ieperen-van Dijk (16) are in agreement with these findings and with our new results.

Huang et al. (29) found weak inhibition of IgE binding to *P. orbiculare* crude extract with *C. albicans* crude antigens. Only a few of the *P. orbiculare* protein components in the highest molecular range exhibited a little inhibition, and no

inhibition of the high-molecular-weight *Candida* mannans was observed with the *P. orbiculare* extract. They suspected that the *P. orbiculare* extract used was rich in protein allergens, but possibly did not contain carbohydrate-rich allergens (29). Several differences between their crude extract and our *P. ovale* crude extract could be found in

Table 1. RAST indexes ([sample – buffer] × 100/[total activity – buffer]) of two AD serum pools and control serum against different yeast extracts without inhibition

	Pool I	Pool II	Control serum
<i>Pityrosporum ovale</i>			
Crude extract	25.4	14	<0.2
Mannan	19.1	8.64	<0.2
<i>Candida albicans</i>			
Crude extract	2.15	3.79	0.7
Mannan	0.77	4.40	<0.2
<i>Saccharomyces cerevisiae</i>			
Crude extract	4.07	7.43	<0.2
Mannan	0.62	1.38	<0.2
<i>Rhodotorula rubra</i>			
Crude extract	0.31	4.02	<0.2
<i>Cryptococcus albidus</i>			
Crude extract	1.11	3.07	<0.2

Table 2. IgG antibodies against different yeast extracts in serum pools analyzed by ELISA (OD) (mean background 0.08 OD is included to values presented)

	Pool I	Pool II
<i>Pityrosporum ovale</i>		
Crude extract	0.62	0.18
Mannan	0.36	0.10
<i>Candida albicans</i>		
Crude extract	1.12	0.52
Mannan	1.87	1.45
<i>Saccharomyces cerevisiae</i>		
Crude extract	0.37	0.16
Mannan	0.94	0.21
<i>Rhodotorula rubra</i>		
Crude extract	0.49	0.11
<i>Cryptococcus albidus</i>		
Crude extract	0.34	0.09

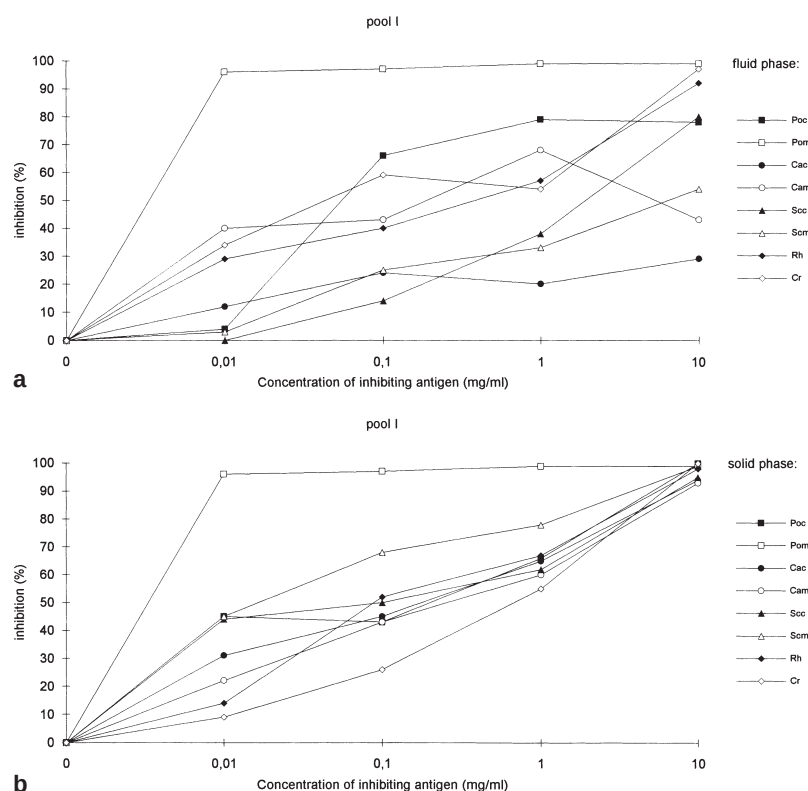


Figure 3. a) Inhibition ELISA of anti-*P. ovale* mannan IgG by fluid-phase incubation in serum pool I with other yeasts; b) inhibition of other yeasts by fluid-phase incubation with *P. ovale* mannan in serum pool I. Percent inhibition was counted from 100% – (sample with inhibition [OD] – buffer/sample without inhibition [OD] – buffer). Symbols are same as in Fig. 1.

the preparation of antigen; for example, culturing times (our 1 week compared to their 4 days), the culture medium used, and sonication compared to our X-press chamber for disruption of the cells. Huang et al. (29) pointed out that Doekes & van Ieperen-van Dijk (16) and Savolainen & Broberg (15) both used commercial *P. ovale* extracts, which seemed to contain only a few protein components, and that would explain some of the differences in the results (29). In contrast, in our *P. ovale* crude antigen, we earlier found 39 different IgE-staining protein bands with also high-molecular-weight mannan staining in immunoblotting (18). Thus, we believe that mannan is an important cross-reacting component of *P. ovale*, but, as Huang et al. (29) pointed out, there is also little inhibition between protein components, as seen also in the present study with the crude antigens.

P. ovale mannan seems to be an important cross-reacting antigen component in IgG binding too. This subject is more complex, since IgG levels against *P. ovale* mannan and crude antigens were clearly lower than against *C. albicans*. IgG

levels of *C. albicans* were high, mannans being even higher than crude extracts. This indicates the importance of *C. albicans* as a trigger for IgG production and also indicates the importance of mannan. Koivikko et al. (1) reported a 6% level of cross-reactivity between sheep and rabbit anti-*C. albicans* IgG antibodies and *P. ovale* extract. Doekes & van Ieperen-van Dijk (16) found $\pm 10\%$ cross-reactivity with commercial rabbit anti-*C. albicans* IgG preparation. Thus, we were the first to use human sera and purified mannan to study the cross-reactivity of IgG-binding components. Our results were different from the two earlier mentioned (1, 16), since we found more cross-reactivity, mannan being important. However, more studies are needed to confirm the cross-reactivity of IgG-binding components.

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