

# A comparison of the development of antibody responses to the polysaccharide antigen (*Candida albicans* mannan) in atopic and healthy infants and children

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## Summary

**Background** Defective antibody response against bacterial polysaccharide antigens is known to be associated with recurrent pyogenic infections. The role of childhood allergy as a risk factor for repeated infections with capsulated micro-organisms has been controversial.

**Objective** To compare the development of polysaccharide specific antibody responses in atopic and healthy infants and children.

**Methods** The antibody responses against a common polysaccharide antigen, *Candida albicans* mannan, were studied longitudinally in 18 atopic and 19 non-atopic children over the first 5 years of life. Determinations of IgA, IgG and IgM antibodies were carried out by enzyme-linked immunosorbent assay and IgE antibodies by nitrocellulose-based radioallergosorbent test.

**Results** The polysaccharide specific antibody responses were similar in both groups, except that anti-mannan IgM levels were higher at 5 years in the atopic children ( $P < 0.05$ , student's *t*-test).

**Conclusion** Atopic children are not more susceptible to bacterial infections on the basis of poorer ability to produce antibodies against polysaccharide antigens.

**Keywords:** *Candida albicans*, mannan, polysaccharide antigen, atopy, infants, children

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## Introduction

Inability to mount a specific antibody response against bacterial polysaccharide antigens is known to be associated with recurrent pyogenic infections even though the levels of serum immunoglobulins are normal [1–3]. *Haemophilus influenzae* type b and *Streptococcus pneumoniae* capsular polysaccharides have been used as antigens for the demonstration of a defect in IgG antibody response to a polysaccharide antigen [4,5]. As most individuals have been exposed to and responded to *Candida albicans*, which is a common commensal

fungus of human microbial flora, cell wall mannan has been suggested to be used as a marker of a response to polysaccharide antigens [6].

In children under 2 years of age the antibody response against bacterial polysaccharides is not fully mature [7,8]. The role of childhood allergy as a risk factor for repeated infections with capsulated micro-organisms has been controversial [9,10]. The present study was undertaken to examine whether the IgA, IgG and IgM antibody responses against a polysaccharide antigen differ in atopic and non-atopic children. Since *C. albicans* cell wall mannan has been shown to act as an allergen at least in adult and adolescent patients with atopic dermatitis and respiratory allergies [11–13], the appearance of IgE antibodies to mannan was also measured.

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**Table 1.** Positive skin-prick test results and diagnoses of the atopic children at 6 months, 18 months and 5 years of age

| Patient |     | 6 months             |    | 18 months                            |         | 5 years                                          |               |
|---------|-----|----------------------|----|--------------------------------------|---------|--------------------------------------------------|---------------|
| No      | Sex | SPT                  | Dg | SPT                                  | Dg      | SPT                                              | Dg            |
| 1       | M   | Egg                  | AD | Egg                                  | AD      | Egg, birch, cat<br>Timothy, <i>Cladosporium</i>  | AD,AB,<br>ARC |
| 2       | F   | Milk                 | AD | Dog                                  | AD      | Dog                                              | AB            |
| 3       | F   | —                    | AD | —                                    | AD,AB?  | Birch                                            | AB?           |
| 4       | M   | —                    | AD | —                                    | AD      | Timothy                                          | ARC           |
| 5       | F   | —                    | AD | Egg,<br>Milk                         | AD      | Egg, birch,<br>Timothy                           | AD,<br>ARC    |
| 6       | M   | Egg                  | —  | Egg                                  | —       | Birch                                            | AB            |
| 7       | M   | —                    | AD | Egg                                  | AD      | Egg, birch, <i>D.p.</i> ,<br><i>Cladosporium</i> | AD,AB,<br>ARC |
| 8       | F   | —                    | AD | —                                    | AD?     | Birch                                            | AD,ARC        |
| 9       | M   | —                    | AD | —                                    | AD?,AB? | —                                                | AB            |
| 10      | M   | —                    | AD | Egg                                  | AD      | —                                                | AB?           |
| 11      | F   | Egg,<br>Milk,<br>Cow | AD | Egg,<br>Milk,<br>Cow,<br>Pea,<br>Soy | AD      | Cat,<br>Cow,<br>Horse                            | AD            |
| 12      | M   | Egg                  | AD | Egg                                  | AD?     | <i>Cladosporium</i>                              | ARC?          |
| 13      | F   | —                    | —  | —                                    | AD      | —                                                | AD            |
| 14      | M   | Egg                  | AD | —                                    | AD?,AB? | Timothy                                          | ARC           |
| 15      | F   | —                    | —  | —                                    | AD?     | —                                                | AD            |
| 16      | M   | Egg                  | AD | Egg,<br>Cat                          | AD,AB   | Egg,<br>Birch,<br>Timothy                        | AB            |
| 17      | M   | Egg                  | AD | Egg                                  | AD      | Birch,<br>Cat                                    | AB            |
| 18      | M   | —                    | —  | —                                    | —       | Birch                                            | ARC           |

M. Male. AD. Atopic dermatitis. AB. Asthma bronchiale ARC. Allergic rhinoconjunctivitis. F. Female. ? Probable disease. *D.p.* *Dermatophagoides pteronyssinus*.

## Materials and methods

### Patients

The mothers of the children had been initially recruited in a study evaluating the effect of maternal exclusion diet in late pregnancy on the development of childhood atopic disease [14,15]. A history of allergic disease, i.e. asthma, rhinoconjunctivitis or atopic dermatitis, was present in at least one family member, i.e. in the mother, father or sibling. The development of allergic disease in the children was monitored over the first 5 years with questionnaires, skin-prick testing, serum-IgE determinations and physical examinations [14,15]. Skin-prick tests (SPT) were carried out at 6 months, 18 months

and 5 years of age. At 6 months, SPT included egg, milk, fish (Dome/Hollister-Stier Laboratories, Slough, UK, 1 : 20 extracts) and pet, if any in the family (Pharmalgen 100 000 BU/mL, Pharmacia, Uppsala, Sweden). At 18 months, in addition to the above mentioned extracts, SPT were performed with the extracts of birch, timothy, house dust mite (*Dermatophagoides farinae*) and mould (*Cladosporium*) (Spectralgen, 10 000 BU/mL, Pharmacia). Histamine chloride 1 mg/mL served as reference. The SPT was regarded as positive if the mean diameter was at least 3 mm for foods and 2 mm for inhalation allergens. At 5 years, SPT consisted of egg, milk, wheat (Dome/Hollister-Stier Laboratories), *Dermatophagoides farinae*, *Dermatophagoides pteronyssinus*, birch, cat, timothy (Pharmacia Diagnostics AB, Uppsala,

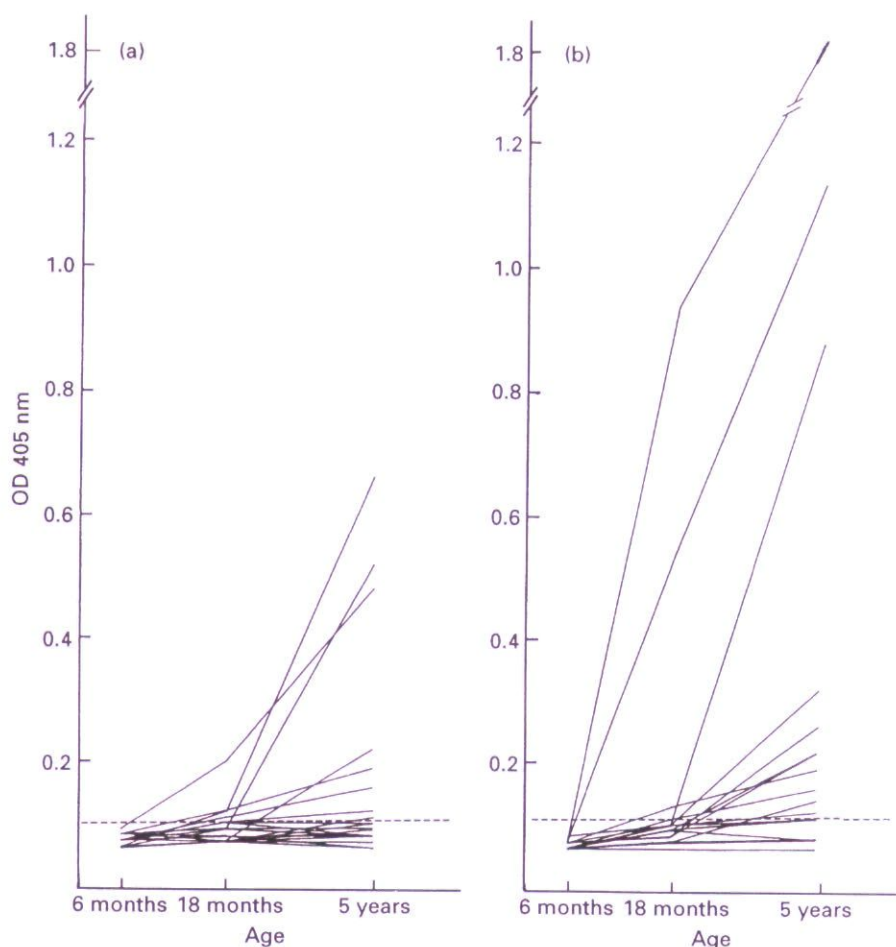


**Fig. 1.** Levels of anti-mannan IgA antibodies in those who did (b) ( $n = 18$ ) or did not (a) ( $n = 19$ ) develop atopic manifestations during the first 5 years of life corresponding to the cut-off level (---).

Sweden). Histamine chloride, 10 mg/mL, served as reference. Serum samples were drawn at the same time points as SPT were performed and stored at  $-20^{\circ}\text{C}$  until analysed. The diagnosis of atopic disease in the child was established by a senior pediatric allergist at an outpatient visit according to the previously described criteria [14,15]. Eighteen children developing an atopic disease and 19 children with no signs of atopic disease, i.e. negative SPT at 6 months, 18 months and 5 years and no history suggesting atopic disease at any of these events, were included in the present study.

#### Antibody assays

For determination of anti-mannan IgA-, IgG- and IgM-antibodies enzyme-linked immunosorbent assay (ELISA) was used. Determination of anti-mannan IgE-antibodies was made with nitrocellulose radioallergo-sorbent test (RAST).

**Antigen extract** The *C. albicans* strain 72024 was supplied and typed by the Mycological Laboratory of the Department of Dermatology, University of Turku, and grown in a fermentor in peptone-supplemented medium as previously described [16]. Mannan was prepared according to the method described by Peat *et al.* where mannan was degraded under alkaline conditions and separated by Fehling's precipitation [17]. The mannan fraction of molecular weight  $> 100\text{ kDa}$  was separated by Amicon ultrafiltration (Amicon Co., Danvers, MA, USA) and used as an antigen in ELISA and RAST.

**Mannan-ELISA** Mannan-specific IgA, IgG and IgM antibodies were measured with ELISA principally as previously described [18]. The wells in polystyrene microtitre plates (Nunc, Roskilde, Denmark) were coated with  $100\text{ }\mu\text{L}$  of  $0.01\text{ mg/mL}$  of dry weight mannan in phosphate-buffered saline (PBS) pH 7.4 and

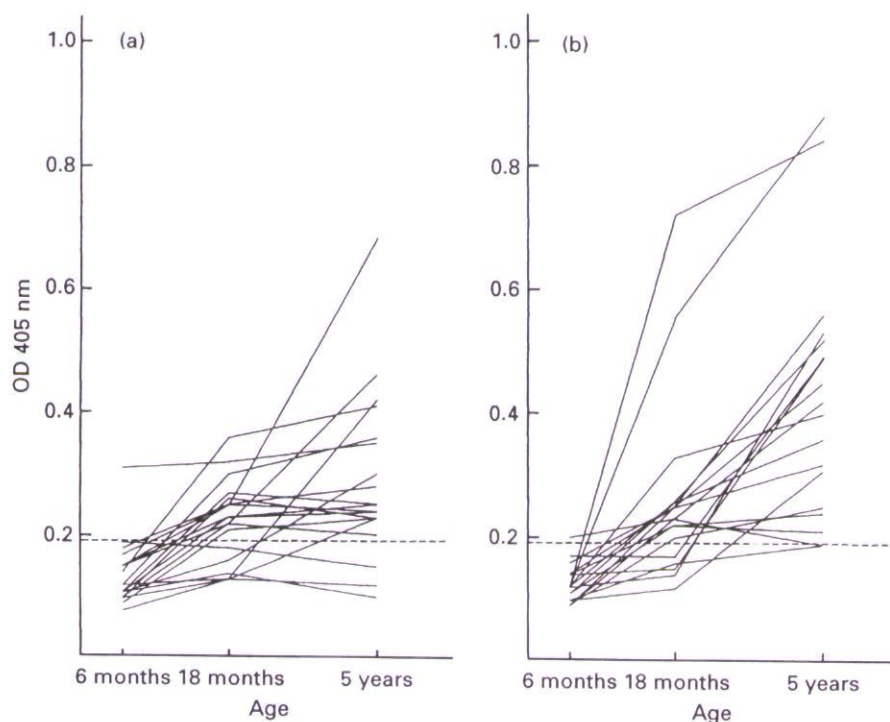


**Fig. 2.** Levels of anti-mannan IgM antibodies during the follow-up period in the same patients as in Fig. 1. (a) Non-atopics. (b) Atopics.

the plates were incubated overnight (16–18 h) at 37°C. This concentration was chosen after testing three anti-mannan IgG positive sera exhibiting the typical high molecular weight mannan stain after SDS-PAGE and immunoblotting [12] against mannan dilutions ranging from 0.001 mg/mL to 0.5 mg/mL of dry weight mannan in PBS. After incubation with the mannan solution, the wells were washed twice with 0.9% NaCl-0.05% Tween (NaCl-T) and thereafter saturated with 1% normal sheep serum in phosphate-buffered saline pH 7.4 (1% NSS-PBS) for 2 h at 37°C. The plates were washed twice with NaCl-T and 75 µL of serum samples, diluted 1:250 in 1% NSS-PBS, were added to the wells and incubated for 2 h at 37°C. Washings were repeated three times and 75 µL of alkaline phosphatase conjugated anti-human-IgA, -IgG or -IgM (Orion Diagnostica, Helsinki, Finland) were diluted 1:250 (anti-IgA and anti-IgM) or 1:500 (anti-IgG) in 1% NSS-PBS and added to the wells and incubated overnight (16–18 h) at room temperature. The plates were washed three times with NaCl-T and 75 µL of *p*-nitrophenyl phosphate in diethanolamine-MgCl<sub>2</sub>-buffer solution (1 mg/mL; Orion Diagnostica) was added and incubated for 25 min at 37°C. The reaction was stopped with 1 M sodium hydroxide. The optical density was measured with a Multiscan Photometer (Labsystems, Helsinki, Finland) at 405 nm. The measurements were made in

duplicate for each serum and arithmetic means were calculated. The intra-assay variation of mannan-ELISA was determined using sera of high, medium and low anti-mannan IgA-, IgM- and IgG-response and was between 3.4 and 10.7%.

**Mannan-RAST** Allergen discs were prepared and mannan-RAST was carried out principally as previously described [13], using the <sup>125</sup>I-labelled-anti-IgE and NaCl-Tween reagents of the commercial Phadebas RAST (Pharmacia, Uppsala, Sweden). In brief, discs of diameter 5.5 mm were cut with an office punch from nitrocellulose sheet (Millipore 0.22 µm, Millipore Co, Bedford, MA, USA). Thereafter, the discs were incubated in antigen solution containing 0.1 mg/mL of dry weight mannan in PBS in a shaker for 1 h at 37°C. Two millilitres of mannan solution were used to coat 25 discs. The discs were washed three times with PBS, air-dried and stored at room temperature. Before use, the discs were rehydrated and the free antigen binding sites were blocked by incubating the discs in PBS-0.5% Tween 20 (PBS-T) in a shaker for 1 h at 37°C. The discs were washed three times with NaCl-Tween. Fifty microlitres of diluted serum (1:2 in PBS-T) was applied onto the discs and incubated in the test tubes for 2 h at 37°C and then the discs were washed three times with NaCl-Tween, 50 µL of undiluted <sup>125</sup>I-labelled anti-IgE (Pharmacia,

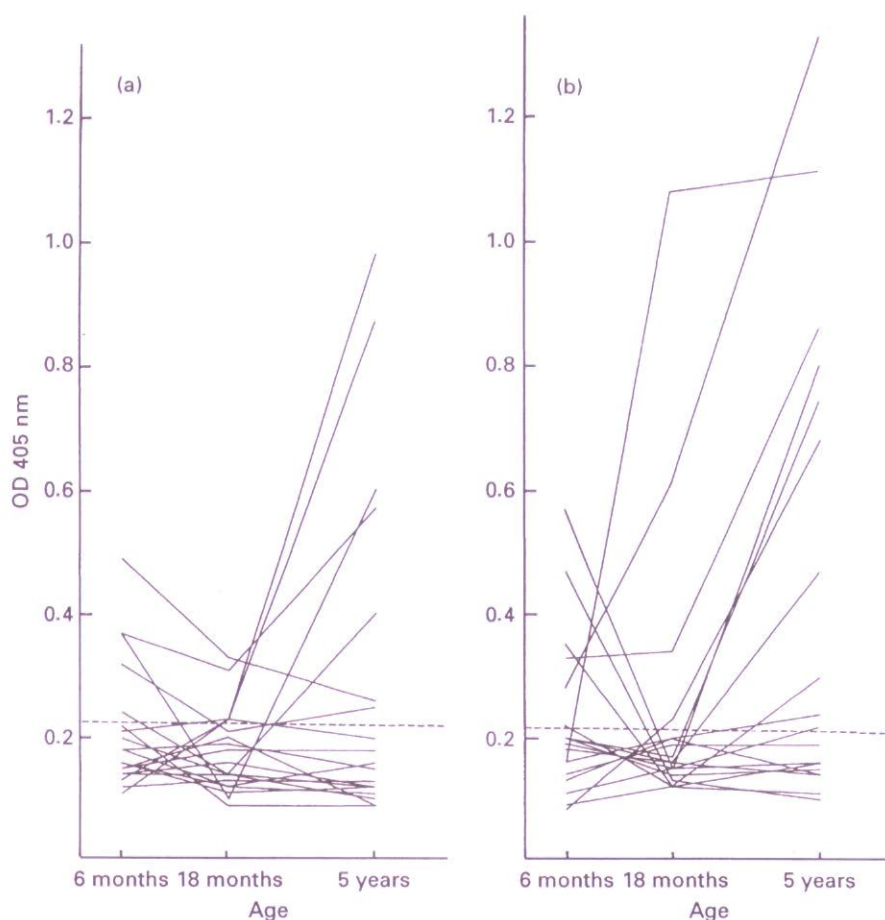


**Fig. 3.** Levels of anti-mannan IgG antibodies during the follow-up period in the same patients as in Figs 1 and 2. (a) Non-atopics. (b) Atopics.

Uppsala, Sweden) was added and the discs were incubated overnight (16–18 h) at room temperature. Maximum total activity was determined in two test tubes containing 50  $\mu$ L of  $^{125}$ I-labelled anti-IgE. The discs were washed three times and bound radioactivity was detected by a gammacounter (1282 Compugamma, Wallac, Turku, Finland). Duplicated determinations were made. Results were expressed as mannan-RAST index values counted as follows:

$$\frac{[\text{sample (cmp)} - \text{buffer (cpm)}] \times 10^2}{\text{total activity (cpm)} - \text{buffer (cpm)}}$$

Intra- and inter-assay variation coefficients were determined using sera of low, medium and high anti-mannan IgE responses and were 8–10.2%.

#### Statistical analysis

Since the results of the antibody assays were normally distributed, the student's *t*-test was used for statistical

analysis. *P*-values < 0.05 were considered to be statistically significant.

#### Results

The characteristics of the 18 children who developed an atopic disease during the observation period are summarized in Table 1. At 6 months, 14 of them had atopic dermatitis, while at 5 years 15 children had a diagnosis of respiratory allergy (asthma or allergic rhinoconjunctivitis), with positive SPT to multiple inhalation allergens and egg.

Anti-mannan IgA and IgM levels increased significantly with age, both in atopic and non-atopic children (Figs 1 and 2). The anti-mannan IgA levels were similar in the two groups at all ages, while mannan-specific IgM antibody levels were significantly higher in the former at 5 years. The anti-mannan IgG levels were similar at 6 and 18 months and at 5 years, both in atopics and non-atopics (Fig. 3). At 5 years the three atopic children with

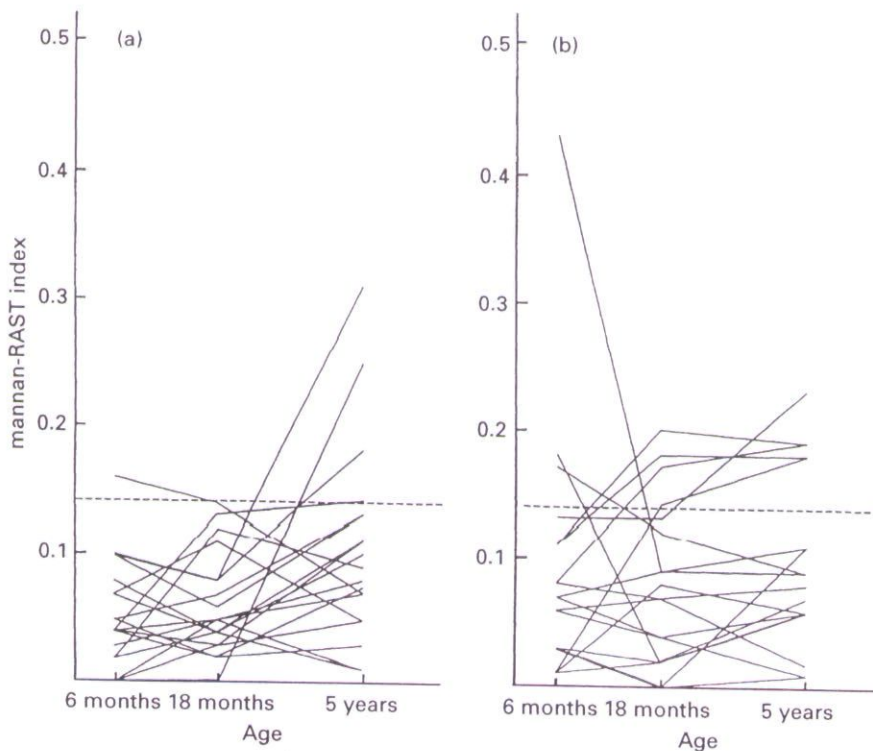


Fig. 4. Mannan-RAST indices at the same time points and in the same individuals as in Figs 1–3. (a) Non-atopics. (b) Atopics.

the high levels of mannan-specific IgA also had the highest values of mannan-specific IgM and IgG.

Since the antibody response against polysaccharides is not fully developed in infancy [7,8], the cut-off levels for the antibody determinations were chosen as upper levels of 95% (mean + 2SD) of the non-atopic infants at 6 months. Five non-atopic infants had anti-mannan IgG above the cut-off, but these antibodies were interpreted to be of maternal origin since the antibody levels decreased in the children by 18 months.

The anti-mannan IgE levels were similar in the atopic and non-atopic children at all ages and the levels did not increase with age (Fig. 4).

## Discussion

The results of the present study showed that IgA, IgG and IgM antibody responses against mannan polysaccharide were similar in atopic and non-atopic children over the first 5 years. Therefore, atopic children might not be anticipated to be more prone to bacterial infections on the basis of poorer ability to produce antibodies against polysaccharide antigens. This is in agreement with studies suggesting that allergy is not associated with recurrent otitis media and infections of respiratory tract [10,19]. However, contradictory results have been

obtained in earlier studies. Kjellman *et al.* [9] reported an overrepresentation of atopic allergy in the group of children suffering from repeated otitis media. Minor *et al.* [20] found a greater frequency of viral respiratory infections among asthmatic children compared with non-asthmatics, whereas no reliable judgement about the occurrence of bacterial respiratory infections could be done due to the higher frequency of antibiotic therapy received by the asthmatics. Immune deficiency might not be the explanation for the greater susceptibility to bacterial infection of an atopic individual, e.g. tissue hyperreactivity and swelling of the mucous membranes have been suggested to lie behind the greater occurrence of otitis media in atopic children [9].

Since no differences were observed in the antibody responses between the atopics and non-atopics, *C. albicans* colonization probably occurs at a similar rate in both groups. Whether the higher level of anti-mannan IgM at 5 years in the atopics suggests increased susceptibility to *Candida* colonization at that age remains unclear even though atopic immune response has been suggested to favour *Candida* growth [21].

IgE-mediated hypersensitivity to *C. albicans* mannan has been shown to be associated especially with atopic dermatitis and to some extent with respiratory allergies in adult and adolescent atopic individuals [11–13]. In the

present study anti-mannan IgE-response remained low during the first 5 years, i.e. during the time when colonization occurred and a primary immune response to the antigen appeared.

The present study showed that the antibody responses against *C. albicans* mannan were similar in atopic and non-atopic children over the first 5 years. As mannan is a representative of a common polysaccharide antigen, we conclude that atopic immune response does not favour susceptibility to bacterial infections via reduced antibody response to polysaccharides.

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