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Slow salivary secretory IgA maturation may relate to low microbial pressure and allergic symptoms in sensitized children

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Abstract

It is unknown why allergic symptoms do not develop in all sensitized children. We analyzed prospectively the postnatal secretory IgA (SIgA) development and whether high SIgA levels would protect sensitized infants from developing allergic symptoms. Salivary total IgA and SIgA levels were determined by ELISA and allergy development investigated at three, six and 12 months and at two and five years in two birth cohorts in Estonia (n=110) and Sweden (n=91), two geographically adjacent countries with different living conditions and allergy incidence. Total and SIgA levels increased with age, reaching adult levels at the age of five. Virtually all salivary IgA in Estonian children was in the secretory form, while a major part of IgA in Swedish saliva lacked the secretory component up to two years of age. In Sweden, high levels of salivary IgA without secretory component correlated inversely with house dust endotoxin levels. High SIgA levels were associated with less development of allergic symptoms in sensitized Swedish children. In conclusion, postnatal maturation of the salivary secretory IgA system proceeds markedly slower in Swedish than Estonian children, possibly as a consequence of low microbial pressure. Secretory IgA may limit allergy-mediated tissue damage at mucosal surfaces in sensitized individuals.

Key words

Allergy

Childhood

Secretory IgA

Endotoxin

Mucosa

Microbial exposure

Saliva

Sensitization

Abbreviation list

EU	endotoxin units
LPS	lipopolysaccharide
pIgR	polymeric immunoglobulin receptor
SC	secretory component
SIgA	secretory IgA
SPT	skin prick test

Introduction

Mucosal surfaces are the main ports of entry for most environmental antigens. The primary humoral mediators of mucosal immunity are secretory IgA (SIgA) and secretory IgM (SIgM), preventing adherence and penetration of antigens through the mucosal epithelium (1). As their polymeric form with an attached secretory component (SC) makes them resistant to cleavage by proteolytic enzymes, they are well suited for surface protection (1). Mucosal SIgA antibody responses are non-inflammatory and are induced by immunoregulatory and IgE-inhibitory cytokines, such as TGF-β and IL-10 (1). An efficient mucosal immune response is a prerequisite for health, since the various mucosal sites are favored as portals of entry by potentially pathogenic antigens and allergens. At birth, only traces of SIgA are found in human exocrine glands, and adult levels are not reached until several years later (2).

Atopic diseases are characterized by high and prolonged circulating IgE antibody responses to allergens (3). A still unsolved conundrum, however, is why some sensitized children do not develop clinical allergy symptoms. We have previously reported that skin prick test (SPT) positive infants without any clinical symptoms of allergy have higher levels of salivary SIgA than those who manifest symptoms (4). Although there is no clear association between mucosal IgA production and development of allergic disease, it is conceivable that high levels of SIgA in the mucosa may prevent allergen absorption, and also possibly interfere with the interaction between allergen and IgE antibodies in sensitized individuals, thereby preventing the development of allergic symptoms. High salivary SIgA levels may also reflect a more mature mucosal immune system (2). Recently, high fecal IgA levels at six months were reported to be associated with a reduced risk to develop IgE-associated disease (5).

The prevalence of allergic diseases has increased considerably in industrialized countries during the last decades (6). Results derived from studies comparing the incidence of atopic diseases in countries of Western and Eastern Europe (7, 8) indicate that the increase is due to

environmental factors associated with an affluent lifestyle. Decreased and/or altered microbial exposure due to *e g* improved hygiene, less infections, antibiotic treatment and altered gut microbiota has been suggested to account for the increase of atopic diseases (9). The latter suggestion is supported by studies showing differences in the composition and diversity of the gut microbiota between allergic and non-allergic infants (10-13), as well as between infants living in countries with a low (Estonia) and high (Sweden) allergy prevalence (14). Moreover, growing up in an environment with heavy continuous external microbial exposure, *e g* farms, reduces the risk to develop allergic diseases (15). Higher levels of lipopolysaccharide (LPS) from the cell wall of gram-negative bacteria were found in house dust collected from farming than from non-farming house-holds (16). Furthermore, we have previously shown higher LPS levels in house dust from Estonian than from Swedish homes, and that high LPS levels were associated with less atopy in Swedish infants (17). It is not known whether early microbial exposure affects the maturation of mucosal IgA responses, however, nor if the postnatal maturation process differs among infants living in different environments.

The aim of this study was to investigate the development of mucosal immunity in Estonian and Swedish children followed from birth up to five years of age, relating the IgA levels in saliva to development of sensitization, allergic disease, infections and environmental endotoxin exposure.

Methods

Subjects

This study comprised 110 Estonian and 91 Swedish children participating in a prospective study regarding development of allergic disease in relation to environmental factors. The study was approved by the Ethics Review Committee on Human Research of the University of Tartu and the Human Research Ethics Committee at the Faculty of Health Sciences, Linköping University. The parents of all children gave their written informed consent. The Estonian children were born during the period February 1997 − June 1998 and the Swedish children were born between March 1996 and March 2000. The children were followed from birth up to five years of age as described in detail elsewhere (18). Briefly, at three, six and 12 months and at two and five years of age the parents answered questionnaires regarding *e g* allergic symptoms, diarrhea, upper and lower respiratory infections, antibiotic treatment and breast feeding (table 1). Furthermore, the children were clinically examined either by a pediatrician or an experienced allergy research nurse, skin prick tests were performed and saliva and blood samples were obtained. Atopic dermatitis was defined as pruritic, chronic or chronically relapsing non-infectious dermatitis with typical features and distribution. Asthma was defined as three or more episodes of bronchial obstruction during the last 12-month period, at least once verified by a physician. Allergic rhinitis/conjunctivitis was diagnosed after appearing at least twice within one hour after exposure to a particular allergen and not related to infections. Urticaria was defined as allergic if it appeared at least twice within one hour after exposure to a particular allergen. The incidence of allergic symptoms is presented in table 2.

Skin prick tests were done at all follow-ups in Estonia, whereas in the Swedish children they were randomly selected to be done either at 3 months in half of the cohort or 6 months in the remaining half of the cohort, and then at 12 and 24 months and 5 years of age. Skin prick tests

were performed in duplicate at the volar aspects of the forearms with fresh skimmed cow's milk and thawed egg white at all follow-ups. At 12 months, the children were also tested with cat allergen extract and in Estonia also with mite allergen extract (*Dermatophagoides pteronyssinus*). At two and five years, birch and timothy were added to the panel, and in Estonia also cockroach (*Blatella germanica*) allergen. All extracts were standardized allergen extracts from ALK (Soluprick®, ALK, Hørsholm, Denmark), except the cockroach extract, which was from Bayer (Spokane, WA, USA). Histamine hydrochloride, 10 mg/ml, was used as positive control and glycerol as negative. The test was regarded as positive if the mean diameter of one of the wheals was at least three mm. The incidence of positive SPT is presented in table 2.

The Swedish children were further categorized as sensitized based on the presence of a positive SPT and/or detectable circulating allergen specific IgE antibodies. This definition is not applicable in the Estonian children, since low levels of circulating IgE antibodies are commonly detected and are poorly related to allergy and SPT positivity (18). The presence of IgE antibodies to egg white and β-lactoglobulin were determined in serum samples collected at all follow-ups up to 24 months, and IgE antibodies to cat and birch allergens were analyzed at 12 and 24 months by a commercial chemiluminescence method, according to the recommendations of the manufacturer (Magic Lite™, ALK). Blood samples were drawn from the Swedish children either at 3 months in half of the cohort or 6 months in the remaining half of the cohort, and then at 12 and 24 months and 5 years of age. Nine Swedish infants were sensitized at three months, 14 at six months, 21 at 12 months and 18 at 24 months. The cumulative number up to that age was 30.

Saliva samples

Non-stimulated saliva samples were collected at all follow-ups from the buccal cavity, using a hand pump connected to a thin plastic tube and immediately frozen and kept at -20ºC. Before analysis, the samples were heated at 56 ºC for 30 minutes and then centrifuged at 5000g for 15 minutes. Reference levels of salivary SIgA were obtained by analyzing samples from 20 healthy adults.

For analysis of total IgA by ELISA, an anti-human IgA antibody directed against the alpha chain (Dakopatts AB, Täby, Sweden) was used as coating antibody, enabling detection of all IgA, including monomeric, polymeric and SIgA. For detection of SIgA by ELISA, an anti-human secretory component antibody (Dakopatts AB) was used, enabling detection of only SIgA. Alkaline phosphatase-conjugated goat-anti-human-IgA antibodies (Sigma Immunochemicals, Stockholm, Sweden) were used for detection. Detailed description of the methods is published elsewhere (4). Human IgA (Sigma Immunochemicals) was diluted to a seven step standard curve, ranging 40-2500 µg/L for total IgA and 16-1000 µg/L for SIgA. The limits of detection were 40 µg/L for total IgA and 32 µg/L for secretory IgA. The inter-assay coefficients of variations (CV:s) were 10% for total IgA and 14% for secretory IgA. Total IgA and SIgA were detectable in all samples.

For detection of IgG antibodies in saliva, a human IgG ELISA quantitation kit was used (Bethyl Laboratories, inc. Montgomery, USA), according to the manufacturer. Human reference serum, containing 4 mg/ml of IgG antibodies, was included in the kit and used as a seven step standard curve, ranging from 7.8 to 500 ng/ml. All saliva samples were diluted 1:10 to 1:1000.

House dust samples

Two dust samples were collected from each household, one from the carpet and one from the child's mattress when the infants were between six and 12 months of age. A dust collector device, containing a 6 µm pore size filter (ALK), was connected to a vacuum cleaner. The

mattress, without sheets, and $2m^2$ of the carpet was vacuum cleaned for 4 minutes. The filters were stored in sterile plastic bags and kept in -20°C until analysis.

The extractions and endotoxin analysis were performed as described in detail elsewhere with a chromogenic Limulus Amebocyte Lysate (LAL) assay (QCL-1000®, Bio Whittaker, Walkersville, ND, USA) (17). The lowest limit for quantitative determinations, for samples diluted 1:1000, was 50 endotoxin units (EU)/ml, corresponding to 0.50 EU/mg dust. Endotoxin analyses were performed on 98 Estonian and 68 Swedish dust samples. The median levels and the range of endotoxin in carpets were 26 (0.5-376) EU/mg in Estonia and 13 (0.5-1393) EU/mg in Sweden, ($p<0.0001$). The corresponding figures for mattresses were 28 (0.5-275) EU/mg in Estonia and 18 (0.5-99) EU/mg in Sweden, ($p=0.004$).

Statistics

As the concentrations of antibodies were not normally distributed, comparisons between unpaired groups were performed with the Mann-Whitney *U*-test and paired groups were analyzed using Wilcoxon signed rank test. Correlations were calculated with Spearman Correlation test. The chi-square test was employed for comparisons of categorical variables. A probability level of <0.05 was considered to be statistically significant.

Results

The levels of total IgA in saliva increased with age, both in Estonian and Swedish infants (fig 1a). The total IgA levels were higher in the Estonian infants at three months, whereas at two years the levels were higher in saliva from Swedish children (fig 1a). At five years, the levels were similar in the two populations (fig 1a).

The levels of SIgA also increased with age in both populations, reaching adult levels at five years (fig 1b). The levels of SIgA were much higher in saliva from Estonian than Swedish children through the first two years of life, however. Similar SIgA levels were observed in Estonian and Swedish children only at five years of age (fig 1b). The similar levels of total IgA and higher levels of SIgA is explained by the fact that most of the salivary IgA in the Estonian infants was SIgA, whereas in the Swedish infants the SIgA levels were markedly lower than the total IgA levels (fig 2). Thus, the SIgA/total IgA ratio was significantly lower in Swedish than Estonian children at 3, 6, 12 and 24 months (Mann-Whitney U-test, $p<0.0001$ for all comparisons), but not at 5 years of age.

Total IgG levels were analyzed in saliva collected at 12 months and at 5 years as a marker of passive transport over the epithelium. At 12 months, the levels of total IgG correlated with the non-SIgA levels in the Swedish infants (*rho*=0.51, $p<0.0001$), but not in the Estonian infants.

House dust endotoxin levels did not correlate with salivary IgA production, except for inverse correlations between the levels in dust from mattresses and carpets and non-SIgA at 6 and 12 months of age in the Swedish infants (*rho*=-0.24-0.48, p=0.01-0.048).

Respiratory infections, *i e* common cold, cough and wheeze, during infancy were more common in Estonian than in Swedish children, except during the first three months of life (table 1). Also diarrhea tended to be more common in Estonia during infancy, whereas the Swedish children tended to have more otitis. The Estonian children were more often treated

with antibiotics during their first 12 months. Respiratory infections between three and six months of age were associated with higher SIgA levels at six months in both Estonian and Swedish children. Similar results were observed for respiratory infections between six and 12 months and SIgA levels at 12 months in the Estonian children (data not shown). Diarrhea, otitis and antibiotic treatment were not associated with salivary IgA levels.

A lower proportion of Estonian than Swedish children attended day care before two years of age (20% vs 79%, $p<0.001$, chi-square test). In Estonia, 4.5% of the children (5/110) lived at 3 months of age in households where both parents smoked as compared to 4.3% in Sweden (4/91, NS). No Estonian and two Swedish children lived in a household where the mother alone smoked (NS). The proportion of children with a smoking father and non-smoking mother was higher in Estonia (35%, 38/110) than Sweden (4.3%, 4/91), $p<0.001$, chi-square test. Day-care attendance and smoking were not related to the IgA and SIgA levels at any age neither in the Estonian nor in the Swedish children, however.

Among the Swedish sensitized infants, those with allergic symptoms during the first two years of life had lower levels of SIgA at 3, 6 and 12 months than similarly sensitized infants without any clinical symptoms, with a similar trend at 24 months of age (fig 3) but not at five years of age (median and interquartile ranges in sensitized children with and without symptoms 141 (90-187) and 176 (134-207) mg/L, respectively). No such association was observed for allergic symptoms at five years in the Swedish children, nor at any age in the Estonian children, possibly due to the low number of children with positive SPT and allergic disease among the latter (table 2). Neither total IgA nor SIgA levels were associated with development of allergic disease or sensitization as such, however (data not shown), although non-secretory IgA (non-SIgA) levels, defined by subtracting the levels of SIgA from the levels of total IgA, were higher in Swedish SPT positive than negative children at six and 12 months of age (fig 4). The non-SIgA levels at 5 years of age were similar in SPT positive and

negative children (median and interquartile ranges 53 (20-100) and 53 (25-88) mg/L, respectively).

Discussion

Mucosal IgA production increased with age, reaching adult levels at five years. In the Estonian children, most of the salivary IgA was in the secretory form at all ages, with a SC attached to the IgA dimer. In contrast, SIgA comprised a much lower proportion of total salivary IgA in the Swedish infants during the first two years of life, indicating that a large proportion of their salivary IgA lacked a SC. At five years of age however, the levels of SIgA and total IgA were comparable also among the Swedish children.

At mucosal sites, such as the salivary glands, dimeric IgA antibodies synthesized in plasma cells beneath the epithelial basement membrane, bind to the polymeric immunoglobulin receptor (pIgR) on the basolateral surface of the epithelial cells (1). The complex is transported to the apical surface, where the pIgR is cleaved to leave the extracellular IgA-binding component bound to the IgA molecule as the so-called SC (1). The pIgR-mediated transocytosis of IgA across the epithelium suggests that all IgA in the lumen of mucosal organs should be in the secretory form. The synthesis of the pIgR may for some reason be delayed in Swedish infants. A reduced pIgR-mediated IgA transport may allow a larger proportion of dimeric IgA to passively diffuse over the epithelium, resulting in the presence of non-SIgA in the salivary gland. Also, a reduced epithelial barrier function may cause increased mucosal leakiness (19). A certain microbial pressure may be mandatory for preservation of an intact mucosal barrier function (20). In support of our findings of non-secretory IgA in saliva of Swedish infants, both monomeric and secretory IgA were present in saliva of Australian children at least up to 12 months of age (21). After 12 months, a switch to only SIgA production was proposed (21)

Total IgG levels in saliva were analyzed at one and five years of age as a marker of non-receptor mediated transocytosis of immunoglobulins over the epithelium. The correlation

between total IgG and non-SIgA levels in the Swedish infants suggests that a large proportion of the IgA in their saliva was secreted without pIgR involvement. This was not the case in the Estonian children.

The expression of pIgR is modulated by microbes and microbial derived products (22) and bacterial LPS and double stranded RNA both up-regulate the expression (23). The concentrations of bacterial endotoxin (i e LPS) in house dust were considerably higher in Estonian than Swedish homes (17). This may possibly explain the higher SIgA levels in the Estonian infants. This suggestion is supported by the inverse relation between house-dust endotoxin levels and the proportion of non-SIgA in the Swedish infants. Furthermore, commensal intestinal bacteria, *e g Bacteroides thetaiotaomicron*, have been shown to up-regulate the expression of the pIgR (24). Pronounced differences in the composition of the gut flora between Estonian and Swedish infants have previously been reported, with a more diverse flora in the former population (14). Moreover, an increased bifidobacterial diversity during the first two months of life was associated with enhanced salivary SIgA levels in Swedish infants (25).

As one of the main functions of SIgA is to protect against mucosal infections by preventing adherence and penetration of microbes (1), low levels of SIgA could theoretically impose an increased susceptibility to infections. In the present study, however, a history of infections early in life were associated with high SIgA levels, probably reflecting the stimulatory effect of the microbial pressure on mucosal immunity early in life.

High levels of salivary SIgA early in life seemed to protect sensitized children from developing clinical signs of allergy. This is in line with a previous report (4). Furthermore, we recently confirmed this observation in a cohort of 200 children followed from birth to four years of age (26). The presence of SC enhances both the stability and effector functions of

IgA (22). High mucosal SIgA levels could possibly interfere with the interaction between allergen and IgE antibodies in sensitized individuals, thereby preventing triggering of allergic inflammation and clinical symptoms. This notion is supported by animal models showing that mucosal IgA responses inhibit airway reactivity in sensitized mice (27-29). Also, induction of IgA to allergens during immunotherapy has been suggested relate to relate to the development of tolerance (30). High levels of non-SIgA may further reflect an immature and leaky mucosal membrane (19), allowing an uptake of allergens through the mucosa. The slow maturation of the salivary SIgA system in Swedish infants was associated with low house dust endotoxin levels and allergy development. This is in line with recent findings that high salivary IgA levels were related to early day-care attendance and less atopy in Australian children (31) and with the hypothesis that the allergy epidemic is caused by a decreased microbial exposure (9).

In conclusion, Swedish children show a markedly slower maturation of the salivary SIgA system than Estonian children, possibly as a consequence of the large differences in microbial pressure between the two countries. Secretory IgA may limit allergy-mediated tissue damage at mucosal surfaces in sensitized individuals.

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Figure legends

Figure 1. Salivary IgA levels (mg/L) in children at three, six, and 12 months and at two and five years of age. 1a) Total IgA levels in Estonian (grey, n=100, 106, 91, 78, 99) and Swedish (white, n=39, 37, 77, 65, 60) children. 1b) Levels of secretory (S)IgA in Estonian (striped, n=101, 106, 91, 78, 99) and Swedish (dotted, n=41, 39, 80, 67, 60) children and from 20 Swedish adults (black). Salivary samples were collected from the Swedish children either at 3 or 6 months, and then at 12 and 24 months and 5 years of age. The 10^{th}, 25^{th}, 50^{th}, 75^{th} and 90^{th} percentiles and outliers (circles) are indicated. **p<0.01, §p<0.001

Figure 2. The total IgA levels (grey in Estonian (Est) and white in Swedish (Sw) children) compared with the SIgA levels in saliva (striped in Estonian and dotted in Swedish children) at three and 12 months of age, n according to figure 1. The 10^{th}, 25^{th}, 50^{th}, 75^{th} and 90^{th} percentiles are indicated as well as outliers (circles). §p<0.001

Figure 3. Secretory (S)IgA (mg/L) in saliva collected at three, six, 12 and 24 months of age from Swedish sensitized infants with (grey, n=9, 5, 13 and 10) and without (white, n=4, 8, 10 and 8) allergic symptoms during the first two years of life. Sensitization was defined as at least one positive skin prick test and/or detection of circulating allergen specific IgE antibodies. The 10^{th}, 25^{th}, 50^{th}, 75^{th} and 90^{th} percentiles and outliers (circles) are indicated. *p<0.05, ¶p=0.11

Figure 4. The levels of non-secretory (S)IgA (the part of total IgA lacking the secretory component), in saliva collected at three, six, 12 and 24 months of age from Swedish infants with positive (n=11, 6, 13, 10) or negative (n=24, 26, 54, 44) skin prick tests (SPT) during the first two years of life. The 10^{th}, 25^{th}, 50^{th}, 75^{th} and 90^{th} percentiles and outliers (circles) are indicated. *p<0.05

Table 1.
The incidence of diarrhoea, respiratory infections, otitis media and antibiotic treatment during the first two years of life, and the length of exclusive and total breast feeding in Estonian and Swedish children. Statistically significant differences are marked with bold.
* = p<0.05, ** = p<0.01, § = p<0.001, Resp. infect. = Respiratory infections.

	0-3 mo	3-6 mo	6-12 mo	12-24 mo	Cumulative 0-24 mo
Diarrhoea					
Estonia	**41/106 (37%)***	32/106 (30%)$^{(p=0.06)}$	59/106 (56%)$^{(p=0.07)}$	58/97 (60%)	90/106 (85%)$^{(p=0.10)}$
Sweden	**20/89 (22%)***	15/83 (18%)$^{(p=0.06)}$	37/83 (43%)$^{(p=0.07)}$	43/83 (52%)	65/86 (76%)$^{(p=0.10)}$
Resp. infect.					
Estonia	**32/110 (29%)****	62/110 (56%)	**99/109 (91%)§**	**95/102 (93%)§**	**109/109 (100%)§**
Sweden	**42/89 (47%)****	38/83 (46%)	**43/86 (50%)§**	**37/83 (45%)§**	**72/87 (83%)§**
Otitis media					
Estonia	No data	No data	24/109 (22%)	**22/101 (22%)***	No data
Sweden	6/90 (7%)	5/84 (6%)	20/86 (23%)	**31/83 (37%)***	44/80 (55%)
Antibiotics					
Estonia	No data	**26/110 (24%)***	**56/109 (51%)****	51/100 (51%)	86/109 (79%)
Sweden	6/88 (7%)	**10/83 (12%)***	**23/83 (28%)****	37/79 (47%)	50/74 (68%)
Breastfeeding	**Exclusive breast feeding (months)**				**Total breast feeding (months)**
Estonia	**2.8 (0.25-6.5)§ (mean (range))**				8.0 (0.5-24) (mean (range))
Sweden	**3.9 (0-8.5)§ (mean (range))**				8.3 (0.25-24) (mean (range))

Table 2.

The incidence of allergic symptoms and skin prick test (SPT) positivity in Estonian and Swedish children followed from birth up to 5 years of age. Clinical evaluations and skin prick tests were done at all follow-ups in Estonia, whereas in the Swedish children they were done either at 3 or 6 months, and then at 12 and 24 months and 5 years of age. Statistically significant differences are marked with bold.

AB; asthma bronchiale, AD; atopic dermatitis, ARC; atopic rhinoconjunctivitis.

* = p<0.05, ** = p<0.01, § = p<0.001

	0-3 mo	3-6 mo	6-12 mo	12-24 mo	Cumulative 0-24 mo	5 years	Cumulative 0-5 years
Allergy							
Estonia	**3/109 (3%)** AD n=3**	**4/108 (4%)§ AD n=4**	**8/109 (7%)** AD n=8**	16/103 (16%)$^{(p=0.06)}$ AD n=11 AB n=6	**18/105 (17%)** AD n=13 AB n=6**	22/98 (22%)$^{(p=0.06)}$ AD n=12 AB n=10 ARC n=7	**24/98 (24%)** AD n=15 AB n=10 ARC n=7**
Sweden	**9/50 (18%)** AD n=9**	**11/49 (22%)§ AD n=10 AB n=1**	**22/90 (24%)** AD n=21 AB n=1**	24/86 (28%)$^{(p=0.06)}$ AD n=21 AB n=5	**28/83 (34%)** AD n=25 AB n=5**	26/73 (36%)$^{(p=0.06)}$ AD n=19 AB n=14 ARC n=13	**34/73 (47%)** AD n=29 AB n=14 ARC n=13**
SPT							
Estonia	5/109 (5%)	**7/108 (6%)****	**7/109 (6%)****	**7/103 (7%)***	**13/107 (12%)***	14/102 (14%)$^{(p=0.09}$	**21/102 (21%)****
Sweden	5/50 (10%)	**10/49 (20%)****	**18/90 (20%)****	**16/86 (19%)***	**22/86 (26%)***	17/69 (24%)$^{(p=0.09)}$	**28/69 (41%)****

Fig 1a Total IgA (mg/L)

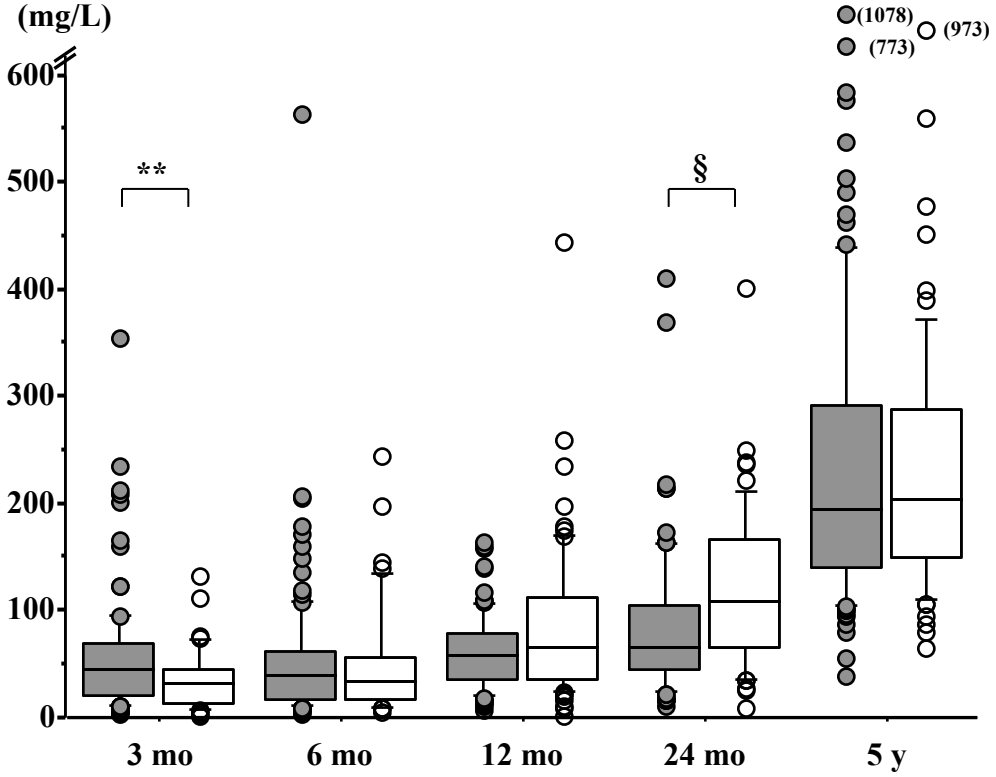


Fig 1b SIgA (mg/L)

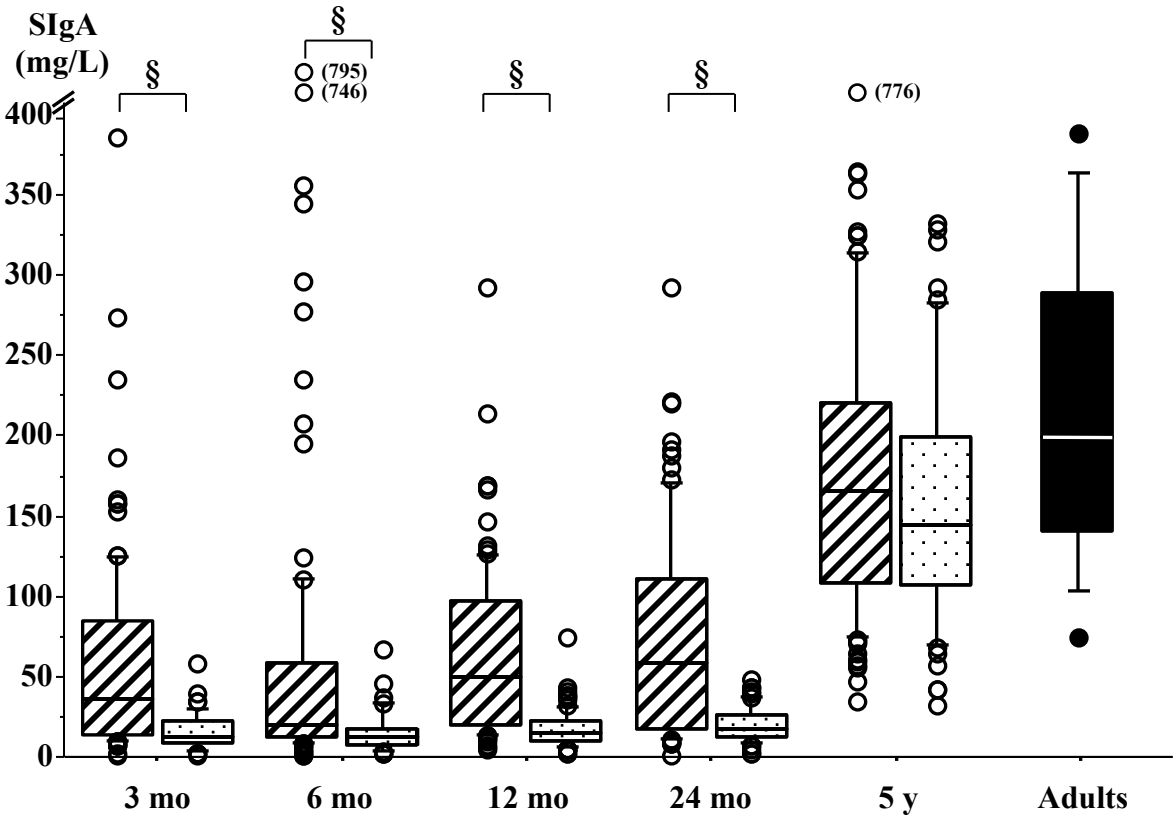


Fig 2 Total IgA and SIgA

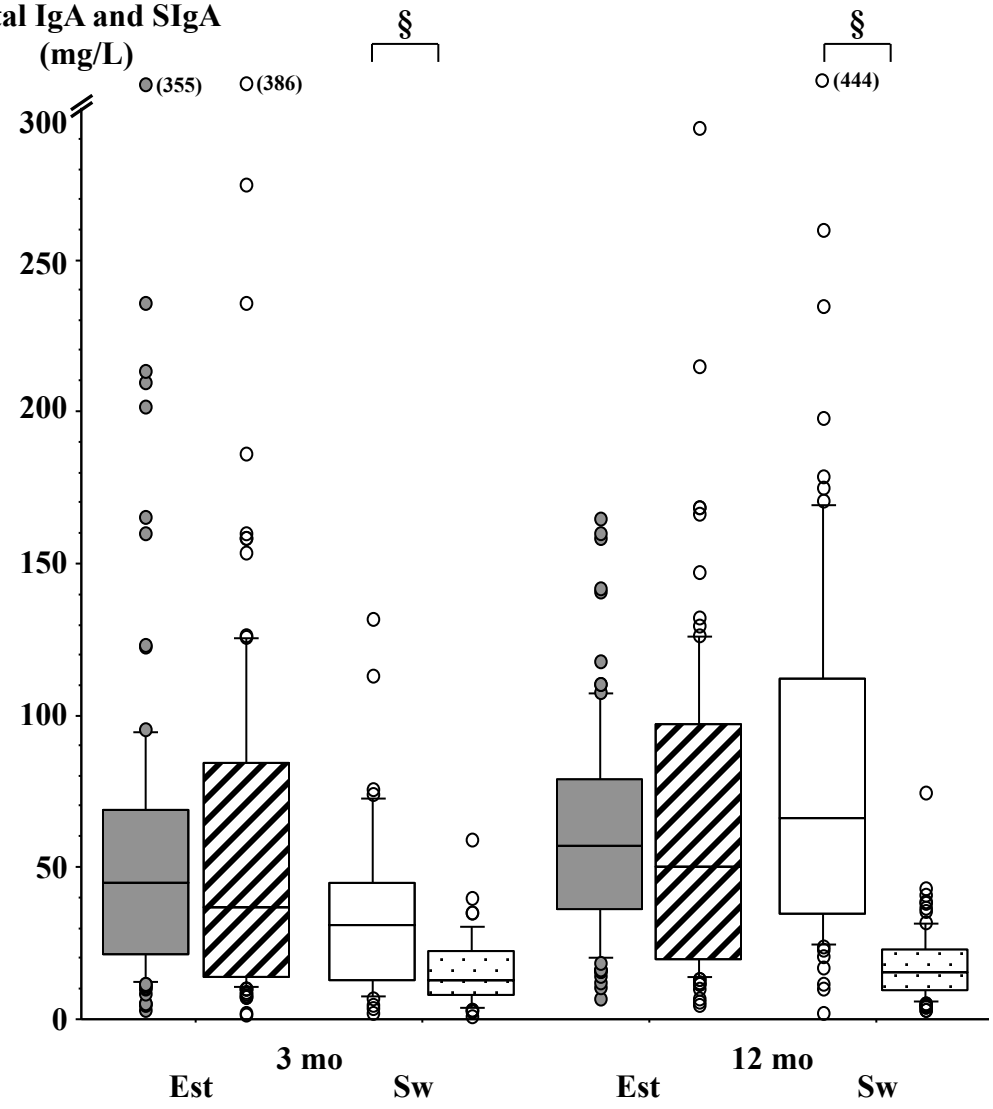


Fig 3

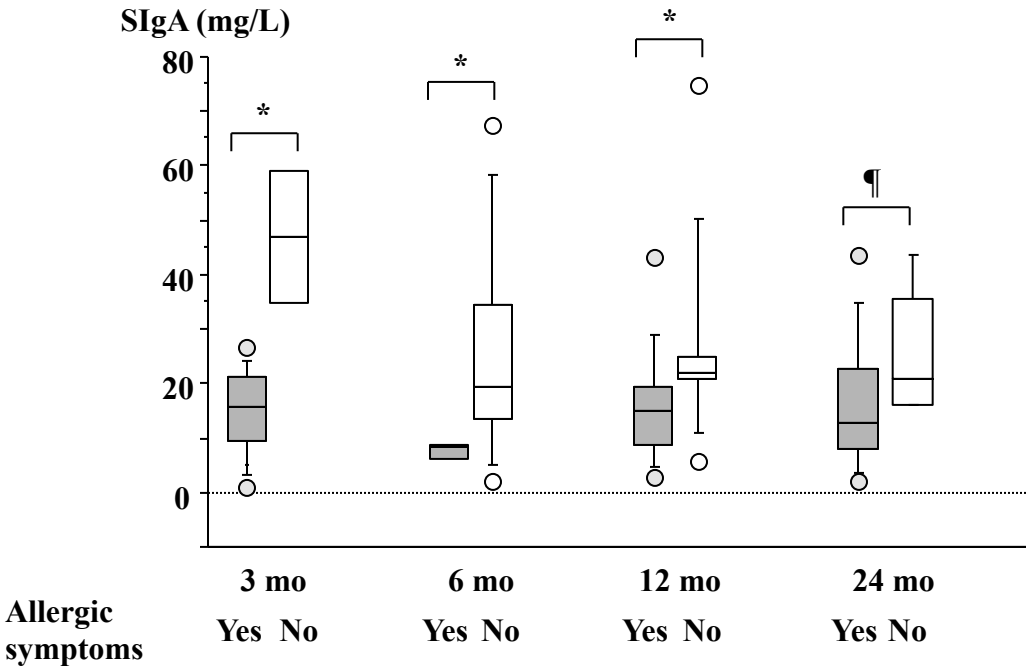
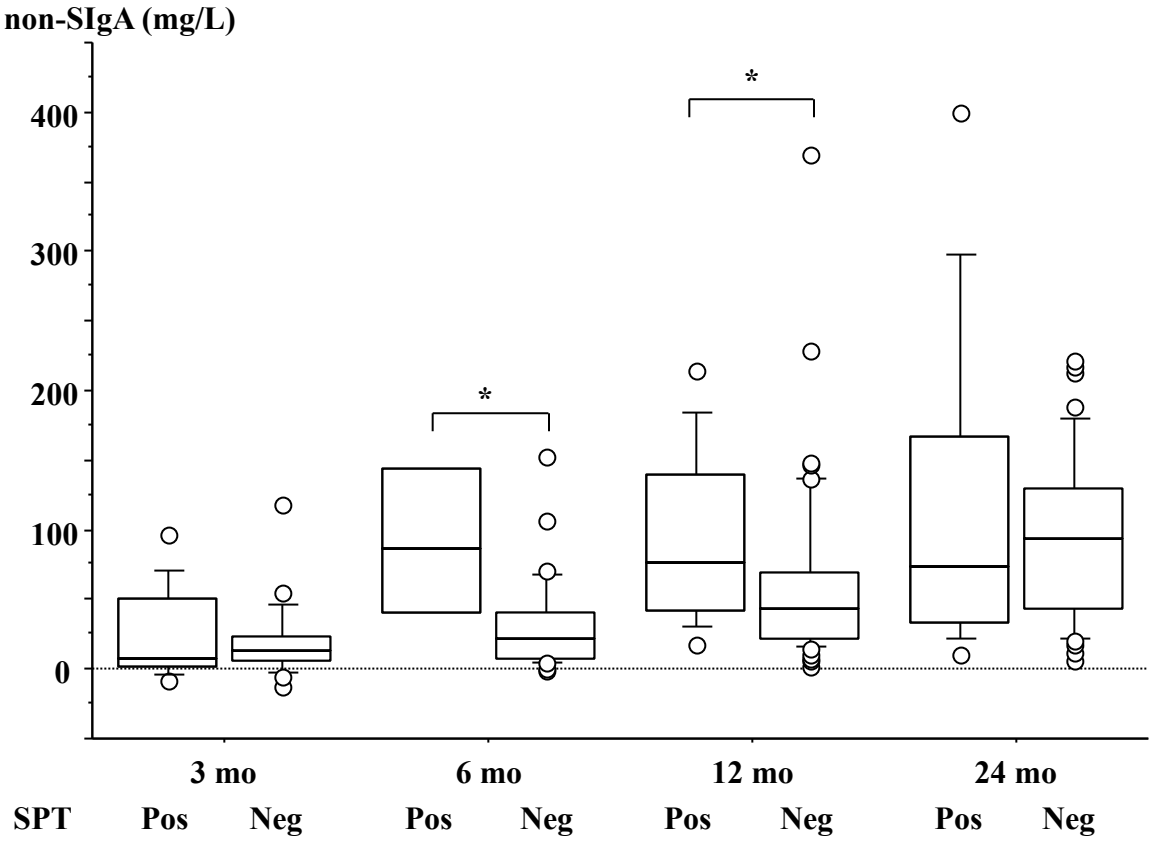

SIgA (mg/L)
80
60
40
20
0
*
*
*
¶
3 mo
6 mo
12 mo
24 mo
Allergic symptoms
Yes No
Yes No
Yes No
Yes No

Fig 4


non-SIgA (mg/L)
400
300
200
100
0
*
*
3 mo
6 mo
12 mo
24 mo
SPT
Pos
Neg
Pos
Neg
Pos
Neg
Pos
Neg