

Allergic diseases and asthma in relation to serum immunoglobulins and salivary immunoglobulin A in pre-school children: a follow-up community-based study

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Summary

Background We have previously reported an association between low IgA and allergic manifestations in early childhood (0–2 years) and have now followed our cohort for an additional 2 years.

Objective To evaluate in a longitudinal community-based cohort study the association between maturation of Ig production and allergic manifestations in the first 4 years of life.

Methods A cohort of 161 randomly selected children was followed from birth to the age of 42–48 months and evaluated at 18–23 months (EV1; $n = 179$) and again at the age of 42–48 months (EV2; $n = 161$). Diagnoses were made with the help of a clinical questionnaire, physical examination and skin prick tests (SPTs) to 10 common allergens. Serum immunoglobulins were measured at EV1 and EV2, and salivary IgA (sal-IgA) at EV2.

Results Serum IgA, IgE, IgG1, IgG2 and IgG4 increased from 2 to 4 years of age ($P < 0.001$) and their levels showed close correlations ($P \leq 0.01$ for most comparisons). Children with one or more positive SPTs had lower serum IgA ($P = 0.004$) and IgG4 ($P = 0.05$) at EV2 than those who did not respond, and children who developed allergic rhinitis between EV1 and EV2 had low sal-IgA ($P = 0.006$) and IgG3 ($P < 0.05$) at EV2. Atopic eczema was associated with low sal-IgA at EV2, and children who developed eczema between EV1 and EV2 had significantly lower sal-IgA than those who recovered after EV1 ($P = 0.02$).

Conclusion Allergic manifestations in predisposed children may be influenced by the rate of maturation of immunological components that counteract sensitization or inhibit effector mechanisms of allergy.

Keywords allergy, asthma, children, immunoglobulins, salivary IgA

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Introduction

Allergy causes significant morbidity in children and several studies have indicated that its prevalence may be increasing, especially asthma [1–4]. The aetiology of allergy is complex, involving polygenic predisposition including major histocompatibility complex genes, dysregulation of T lymphocyte function and various environmental factors [2–6]. IgE antibodies play an important effector role in atopic allergy, but there is increasing evidence that T cells and cytokines are also involved, especially in late-phase reactions [6–8]. Furthermore, a variety of immunological components, including non-IgE antibodies, may have a protective or disease-modifying function in allergy-prone individuals. Thus, over 50% of patients with IgA deficiency have been reported to be atopic [9, 10], and low concentration or deficiency of IgG subclasses has been associated with asthma [11–13]. Allergic diseases are heterogeneous, and may

conceivably be caused by different underlying mechanisms depending, e.g. on the age of onset [14]. Our previous results have indicated that the frequency and severity of allergic manifestations in children around 2 years of age is more closely associated with low normal serum IgA (lowest quartile) than with raised IgE [15]. Recurrent otitis media and respiratory tract infections were also more common among children with low normal serum IgA [15, 16]. The aim of our present study was to further evaluate the association between maturation of Ig production and allergic manifestations by following up our cohort for an additional 2 years; in this second phase of our study, we report on our findings concerning the interplay of immunoglobulins and allergy in this cohort of children when they were 42–48 years old.

Materials and methods

Patients

The study was approved by the Ethical Committee of Landspítali University Hospital. The first phase of the study served to evaluate the predictive value of cord blood IgE for

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atopy and related disorders and involved measurement of IgE and IgA in samples taken from infants at delivery ($N = 792$) and again at 2 years of age ($N = 179$) [15, 16]. The second phase, reported here, serves to evaluate the association between maturation of Ig production and allergic manifestations in the first 4 years of life. Of the 179 children evaluated in 1989 (EV1) at the age of roughly 2 years (18–23 months), 161 children (90%) participated 2 years later (EV2) when they were about 4 years old (42–48 months). Informed consent was obtained from the parents. Clinical manifestations between these two assessments were recorded separately and compared with those recorded at EV1. As before [15], otitis media was only recorded if it had been diagnosed by a physician. Similarly, asthma was recorded only when three or more wheezing attacks had been diagnosed as asthma by a physician. No attempt was made to distinguish between allergic and non-allergic asthma in this study. Skin prick tests were carried out with the following 10 components that are very common allergens in Icelandic children: cow's milk, egg white, cod, beans, wheat, cat and dog hair, dust mite (*Dermatophagoides pteronyssinus* and *D. farinae*) and grass (*Phleum pratense*). A test was considered definitely positive if the reaction was equal or greater than the histamine control (3+) and suggestive if it was clearly present but smaller (2+). The clinical findings recorded for each child were evaluated by a paediatric allergist who did not know about the laboratory findings, and each child was given a global allergy-severity score [15] taking into account the number of wheezing attacks (0–5), the severity or persistence of atopic eczema (scored 0–5), immediate skin reaction to one or more allergen (scored 0–2), history of allergic reaction to food (scored 0–2) and urticaria (0–1). Children scoring 4 or more were considered to have definite allergy.

Materials

Blood samples were allowed to clot at room temperature, the sera aliquoted and kept at -70°C . Unstimulated saliva was collected with plastic Pasteur (Volac, Barking, UK) pipettes 3 h postprandially and maintained at -70°C . Rabbit anti-human α -chain (code A262), alkaline phosphatase (AP)-conjugated rabbit anti-human IgA (code D338), F(ab)2 rabbit anti-human IgE (code A094) and AP-conjugated rabbit anti-mouse IgG (code D314) were purchased from Dako (Copenhagen, Denmark), mouse anti-human IgE (I-339-03/04) from Medix (London, ON, Canada), IgA standard from Beckman-Coulter (Fullerton, CA, USA), IgE standard from Pharmacia Diagnostics (Uppsala, Sweden) and IgG subclass-specific sheep antibodies from Sanquin (Amsterdam, the Netherlands).

Serum and salivary IgA was measured by a sandwich ELISA [16] using rabbit anti-human α -chain for coating and AP-conjugated rabbit anti-human IgA for developing. Samples were diluted 1/3200 in phosphate-buffered saline-Tween (sera), or used undiluted (saliva). The absorbance of each sample was read against dilution curves of a standard with a known concentration of IgA. The within-assay variability was 10% and the between-assay variability did not exceed 15%. This was true for both serum and salivary IgA. The detection limit was 0.023 g/L.

Serum IgE was measured by ELISA [16], using F(ab)2 rabbit anti-human IgE for coating and mouse anti-human IgE followed by AP-conjugated rabbit anti-mouse IgG for developing. Samples were compared with serial dilutions of an IgE standard. The detection limit of the ELISA was 0.23 KU/L. The within-assay variability was 10% and between-assay variability did not exceed 20%.

IgG subclasses were measured by radial immunodiffusion using subclass-specific sheep antibodies and a standard calibrated against WHO Ig Reference preparations 67/97 [17]. The within-assay variability was 10% and the between-assay variability did not exceed 15%. The detection limit was 0.28 g/L for IgG1, 0.15 g/L for IgG2, 0.04 g/L for IgG3 and 0.06 g/L for IgG4.

Data analysis

In addition to general descriptive statistics, groups were compared by *t*-test, Mann–Whitney or Fisher's exact statistics and correlated with the Spearman's test. Significance was set at $P < 0.05$.

Results

Age distribution of immunoglobulin levels

Levels of immunoglobulins in the cohort are shown in Table 1. One child had IgA-deficiency. Regardless of medical history, a significant (or near-to-significant) age-associated increase was observed in all Ig classes and subclasses.

Correlation between immunoglobulin classes and subclasses

Levels of each Ig class were significantly correlated between the different ages (Table 2). Weak-to-moderate correlation was also seen between most Ig classes and subclasses, especially at 2 years of age.

Comparison of clinical findings at EV1 and EV2

By the age of 2, 37% of the children had developed moderate or severe allergy and the prevalence remained similar (31%) 2 years later (Table 3). As expected, more children presented with atopic eczema than with asthma between the age of 2 and 4. Although the overall prevalence of atopic eczema did not decrease, fewer children had severe eczema at EV2.

Table 1. Median levels and ranges for serum immunoglobulins measured by ELISA

	Median (range) g/L		$P^{\dagger}$
	2 years*	4 years†	
IgG1	6.1 (3.2–12.4)	7.0 (3.5–15.5)	<0.001
IgG2	0.9 (0.26–7.5)	1.1 (0.19–3.5)	<0.001
IgG3	0.26 (0.07–1.0)	0.28 (0.07–0.8)	0.064
IgG4	0.11 (0.01–1.7)	0.21 (0.06–1.0)	<0.001
IgA	0.61 (0.04–2.9)	0.82 (0.03–2.1)	<0.001
IgE	0.1 (0.1–6.4)	0.73 (0.2–39)	<0.001

*IgE at birth.

†IgE at 2 years.

‡Mann–Whitney.

Table 2. The Spearman correlation coefficient and statistical significance for serum and salivary (sal-) IgA at birth (0), 2 years (2) and 4 years (4) of age and for maternal serum (m) at the 2-year visit

Correlations	<i>r</i>	<i>P</i>	Correlations	<i>r</i>	<i>P</i>
IgE (0) vs. IgE (2)	0.32	<0.01	IgG1 (2) vs. IgG3 (4)	0.19	0.02
IgG1 (2) vs. IgG1 (4)	0.40	<0.01	IgG3 (2) vs. IgG1 (4)	0.19	0.02
IgG2 (2) vs. IgG2 (4)	0.64	<0.01	IgG4 (2) vs. IgG2 (4)	0.24	<0.01
IgG3 (2) vs. IgG3 (4)	0.64	<0.01	IgG4 (2) vs. IgA (4)	0.24	<0.01
IgA (2) vs. IgA (4)	0.39	<0.01			
IgA (2) vs. IgG1 (2)	0.31	<0.01	IgG1 (4) vs. IgG2 (4)	0.19	0.02
IgA (2) vs. IgG2 (2)	0.26	<0.01	IgG1 (4) vs. IgA (4)	0.22	0.01
IgA (2) vs. IgG3 (2)	0.26	<0.01	IgG2 (4) vs. IgG3 (4)	0.21	0.01
IgA (2) vs. IgG4 (2)	0.18	0.02			
IgA (2) vs. IgE (2)	0.17	0.03	IgG2 (2) vs. sal-IgA (4)	0.22	0.01
IgG1 (2) vs. IgG2 (2)	0.31	<0.01	IgG2 (4) vs. sal-IgA (4)	0.22	0.01
IgG1 (2) vs. IgG3 (2)	0.42	<0.01	IgA (4) vs. sal-IgA (4)	0.20	0.01
IgG1 (2) vs. IgG4 (2)	0.17	0.04			
IgG2 (2) vs. IgG3 (2)	0.25	<0.01	IgE(m) vs. IgG3 (2)	0.19	0.02
IgG2 (2) vs. IgG4 (2)	0.38	<0.01	IgE(m) vs. IgG4 (2)	-0.23	0.01

Table 3. Clinical events recorded between birth and 2 years (0–2) and between 2 and 4 years (2–4) of age

	Numbers (%)	
	0–2 years (<i>n</i> = 179)	2–4 years (<i>n</i> = 161)
Non-allergic*	108 (60)	98 (61)
Possible allergy*	5 (3)	13 (8)
Moderate allergy*	42 (24)	34 (21)
Severe allergy*	24 (13)	16 (10)
Asthma	23 (14)	26 (16)
Atopic eczema (total)	31 (19)	42 (26)
Severe eczema	11 (7)	3 (2)
Allergic rhinitis	0 (0)	12 (7)
Food allergy	2 (1)	0 (0)
Otitis media > 2 attacks	121 (75)	72 (45)
Otitis media > 4 attacks	48 (27)	22 (14)

*Non-allergic = allergy severity score < 3; possible allergy = score 3; moderate allergy = score 4–5; severe allergy = score > 5 [13].

Table 4. Recovery and onset of allergic disease at age 2–4*

Allergy at EV2	Not allergic at EV1	Allergic at EV1
No (<i>n</i> = 98)	81	17
Yes (<i>n</i> = 50)	16	34

*Children attending at both EV1 and EV2 with a definite diagnosis (excluding 13 with possible but doubtful allergy at EV2 and five at EV1).

Allergic rhinitis (AR) was not present until after the age of 2 in our cohort, but of the 12 children who had AR by EV2 nine had some other allergic manifestations at EV1.

A closer look at children with a definite diagnosis of allergy (Table 4) showed that out of the 51 children with allergy at EV1, 17 (33%) had recovered by the age of 4 while 16 (16%) of the 97 children who were not allergic at EV1 had developed allergy by the time of EV2.

Table 5 shows the clinical manifestations observed at the 4 years examination. Allergic manifestations and otitis media, previously shown to affect a high proportion of children in the preceding 2 years (cf. Table 3), could be verified at evaluation in quite many children.

Table 5. Clinical manifestations present at EV2

Diagnosis	% of cohort (<i>n</i> = 161)
Atopic eczema (total)	18.0
Acute otitis media	5.6
Serous otitis media	14.3

Skin prick tests and serum immunoglobulins

At EV2, 25 children (16%) had a positive reaction to one or more of the 10 allergens tested, of whom 18 (70%) were diagnosed with clinically relevant allergic diseases. As expected children with allergy were more likely to show positive reaction to one or more allergen than the non-allergic children. Children with atopic eczema did not react preferentially to any of the allergens tested (data not shown). The skin test-positive children had lower levels of serum IgA ($P = 0.004$) and IgG4 ($P = 0.05$) at EV2 (Fig. 1) than the skin test-negative children but levels of serum IgE and other Ig subtypes were similar.

Clinical manifestations and immunoglobulin isotypes

At the age of 2, allergy was more common among children with relatively low levels of serum IgA (lowest quartile) and at this age severe allergy showed a stronger correlation with low levels of IgA than with high levels of IgE [15]. At the age of 4, an overall association between allergy and low levels of serum IgA was no longer present (Table 6). This was mainly because of the asthmatic children who, in contrast to having low serum IgA levels at the age of 2, had relatively high levels of serum IgA at the age of 4, and this also applied to salivary IgA (Table 6). Children with the more recently diagnosed AR had lower levels of serum and salivary IgA and this was significant for salivary IgA (sal-IgA) at EV2 (Table 6, Fig. 2). A weak but significant correlation was observed between serum and salivary IgA ($\rho = 0.2$, $P = 0.015$). Children with AR also had lower levels of serum IgG2, IgG3 and IgG4, and this was significant for IgG3 (Table 7).

The prevalence of atopic eczema was higher in children with low than high sal-IgA (lowest vs. highest quintile) (Table 6)

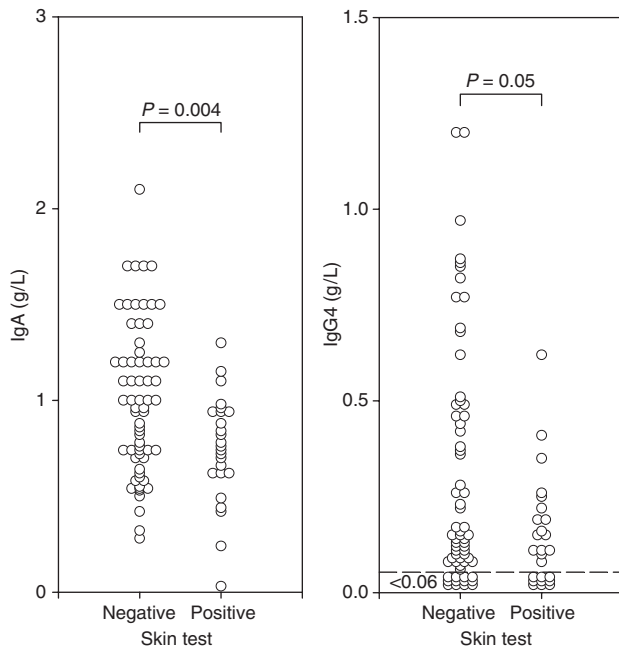


Fig. 1. Serum values of IgA and IgG4 in skin test-positive ($n = 25$) and skin test-negative ($n = 133$) children. Statistical analysis: t -test (IgA; power 0.811); Mann-Whitney U -test (IgG4).

Table 6. Prevalence of allergic diseases (%) at the age of 4 in children in the lowest and the highest quintile of serum and salivary IgA (sal-IgA) and serum IgE

	Serum IgA (g/L)		sal-IgA (g/L)		IgE (KU/L)	
	<0.59	>1.19	<0.028	>0.067	<0.16	>2.65
Asthma (%)	18.2	33.3	18.8	36.4	15.0	30.0
Allergic rhinitis (%)	12.5	3.0	15.6	0.0*	7.5	10.0
Eczema (%)	37.5	21.2	40.6	18.8	32.5	32.5

* $P = 0.05$ (Fisher's Exact).

and sal-IgA was significantly lower in children who developed eczema between EV1 and EV2 ($n = 18$) than in the children who recovered from their eczema after EV1 ($n = 19$) (medians 0.036 vs. 0.054 g/L, $P = 0.017$).

Discussion

It has previously been reported that about 37% of Icelandic children participating in a long-term study had developed definite allergic manifestations when first evaluated at the age of 18–23 months. At this age allergy, including asthma, was most prevalent in children in the lowest quartile of serum IgA, and children with high IgE seemed to be protected if their IgA was relatively high [15]. None of the children had been treated with systemic steroids. The present report describes a follow-up evaluation when these children were 42–48 months old. Of the 179 children who participated in the first part of the study (EV1), 161 (90%) could be recruited for the follow-up (EV2). The incidence of definite allergic manifestations remained high but had declined from 37% to

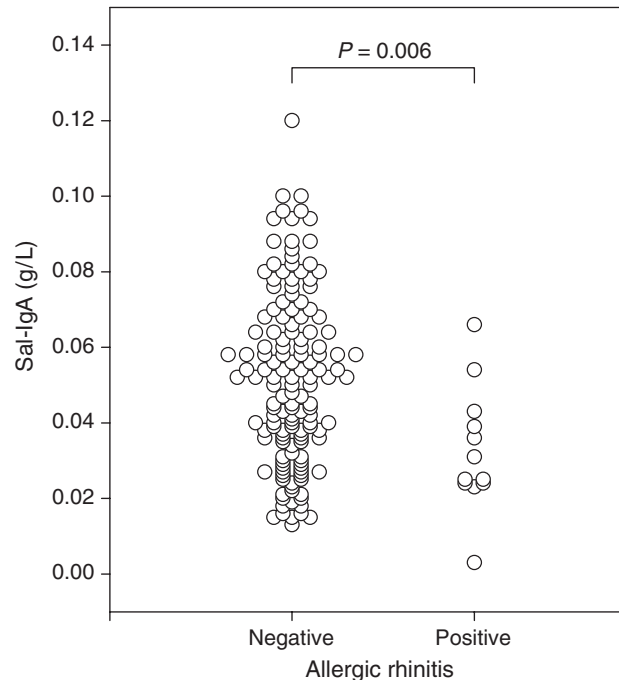


Fig. 2. Salivary IgA (sal-IgA) in children with ($n = 12$) and without ($n = 149$) allergic rhinitis. Statistical analysis: t -test (power 0.75).

Table 7. Prevalence of allergic diseases (%) at the age of 4 in children in the lowest and the highest quintile of IgG subclasses

	IgG1 (g/L)		P^*	IgG2 (g/L)		P
	<5.21	>9.29		<0.69	>1.64	
Asthma (%)	17.2	46.9	NS	28.1	21.2	NS
AR (%)	10.3	6.3	NS	15.6	3.0	0.1
Eczema (%)	27.6	21.9	NS	40.6	24.2	NS
	IgG3 (g/L)		P^*	IgG4 (g/L)		P
	<0.18	>0.41		<0.06	>0.45	
Asthma (%)	20.7	27.6	NS	32.4	30.3	NS
AR (%)	13.8	0.0	<0.05	16.2	3.0	0.11
Eczema (%)	10.3	22.2	NS	32.4	12.1	0.08

*Fisher's Exact.

NS, non-significant, AR, allergic rhinitis.

31% during the 2 years between EV1 and EV2. As could be expected, the majority of the children who were allergic at the age of 4 years (EV2) had also been allergic at EV1 (34 out of 59, Table 4). Thus, 16% of the cohort was still affected by asthma (Table 3) and nearly half of these children were symptomatic at EV2 (Table 5).

The high incidence of allergy that we observed agrees with the general notion that atopy-related diseases are becoming increasingly common [1, 2]. The incidence in our cohort of 31% is similar to recently published values in Sweden [1] but both lower and higher values have been reported [18, 19]. It should be pointed out in this context that pollution is very low even in urban areas of Iceland.

At 4 years of age, the previously observed association between asthma and low normal serum IgA (lowest quartile

at 2 years of age) was no longer apparent; it should be noted that by 4 years all but one of the children had acquired IgA serum concentration above the lowest quartile for the 2-year-old. At EV2, asthma was in fact associated with relatively high IgA, probably reflecting a persistent antigenic stimulation of the respiratory mucosa. Thus, while low concentration of IgA may facilitate sensitization of predisposed children, relatively high IgA levels do not seem to prevent asthmatic attacks in children who have already been sensitized. Saliva was not collected at EV1 but children who developed AR between EV1 and EV2 had significantly reduced sal-IgA at EV2. Moreover, children with eczema of recent onset had significantly lower sal-IgA at EV2 than those who had recovered. Both these findings agree with recent observations [20–23] and transient absence of sal-IgA in infancy has also been associated with increased risk of developing bronchial hyper-reactivity later in life [24]. Taken together, these findings suggest that young children (≤ 4 years) who are genetically predisposed to allergy are more readily sensitized if their IgA is in the lower range of normal, and the same might apply to IgG3 (the present study; also see [11]), IgG2 and IgG4 [12]. At least two not mutually excluding explanations might account for these findings. (1) The non-IgE antibodies might help to neutralize potential allergens both at the mucosal and systemic level, by combating viral infection, which is thought to facilitate allergic sensitization in early infancy [4], and/or by a 'blocking' effect [25]. It may be relevant in this context that an increased gut permeability [26–28] and decreased clearance rate of ingested proteins [29] have been demonstrated in patients with atopic dermatitis. Thus, at a critical time-point, children low in IgA and certain IgG subclasses could be at risk of allergic sensitization because their mucosal barriers are impaired. (2) Alternatively, the association between allergy and low concentration of non-IgE antibodies might reflect aberrant T cell regulation at mucosal surfaces, leading to deficient B cell maturation on the one hand and allergic sensitization on the other. Both IgA levels and allergic sensitization could thus be affected by deficient transforming growth factor (TGF)- β responses as this cytokine not only stimulates IgA production but may also indirectly inhibit proliferation of Th2 cells [30] whose cytokines may play a key role in the early pathogenesis of atopic disease [7–8]. Similarly, IFN- γ and IL-2 influence antibody production and class switching [13]; decreased production of these cytokines has been reported in infants with subsequent development of atopy [31, 32]. Finally, recent observations indicate that IL-4 and IL-10 may induce switching of naïve B cells not only to IgE but also to IgG1 and IgG3 [33, 34], and this observation could in fact explain our finding of increased IgG1 and IgG3 in children with atopic dermatitis. Whether non-IgE antibodies are directly involved in allergy pathogenesis or reflect sensitizing circumstances because of a cytokine imbalance remains to be studied. Most cases of IgG subclass deficiency appear to result from a regulatory dysfunction, and levels usually normalize later in life [35, 36]. It seems tempting to speculate that this reflects aberrant T cell regulation, and this could also lead to low levels of IgA and sal-IgA [37]. However, the question remains whether atopic disease is caused directly by T cell imbalance or whether the effect is indirect, through a deficit in 'blocking' non-IgE antibodies.

It is concluded that atopic predisposition is regulated by dynamic and complex processes involving many factors that may either promote or suppress clinical symptoms, and that these mechanisms may be more delicately balanced during the maturation of the immune system in early childhood. Additional longitudinal studies are needed to further clarify the physiological factors that could eventually be modulated to counteract allergic sensitization or manifestations.

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