

BRIEF COMMUNICATION

Serum allergen-specific IgA is not associated with natural or induced tolerance to egg in children

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Abstract

The role of specific IgA (sIgA) in oral immunotherapy (OIT) and natural tolerance to foods is poorly understood. We aimed to study serum sIgA in induced and natural tolerance to egg. Children aged 5–16 years diagnosed with IgE-mediated egg allergy were recruited. After egg challenge, patients were classified as transient (TEA) or persistent (PEA) egg-allergic. PEA children were further divided into oral immunotherapy (PEA-OIT) or egg avoidance (PEA-EA). Allergy/tolerance was reassessed 9–12 months later (T1) in PEA-EA. Serum sIgA to ovalbumin and ovomucoid were determined at inclusion in all patients and repeated in PEA at T1. 21 TEA and 52 PEA children were recruited (28 PEA-OIT, 24 PEA-EA). Serum sIgA remained unchanged after OIT. TEA and PEA had similar serum sIgA. No specific trend on serum sIgA was observed in five PEA-EA who developed natural tolerance over follow-up. Thus, serum sIgA seems not to be associated with induced or natural egg tolerance.

Secretory IgA, as the major antibody at mucosal sites (1), seems to play a key role in oral tolerance by preventing antigen uptake through the intestinal epithelium (2–5). Indeed, high secretory IgA in faeces (4) and saliva (6) has been associated with a reduced risk of allergic diseases. Moreover, recent studies provided promising results involving specific IgA (sIgA) in allergen tolerance. In a murine model of oral tolerization to beta-lactoglobulin, Peyer's lymphocytes were identified as key regulators of clinical tolerance, leading to increased secretory sIgA, whereas serum sIgA remained low (2). In contrast, the potential role of serum sIgA as a protective mechanism in food allergy was recently highlighted in egg-allergic mice (7), as sIgA suppressed anaphylaxis by neutralizing the absorbed antigen in the circulation rather than by preventing the antigen uptake in the intestinal mucosa. Oral immunotherapy (OIT) is a promising experimental treatment for IgE-mediated food allergy. Immunological mechanisms involved in OIT might be paralleling those underlying spontaneous tolerance development, but these mechanisms are still poorly understood (8). Regarding sIgA and OIT, Leonard reported increased serum sIgA in mice after egg OIT (8), paralleling previous results of aeroallergen immunotherapy (9, 10). In children under peanut sublingual immunotherapy (SLIT), increase in

serum and salivary sIgA was reported, as well as increased salivary secretory IgA (3). Nonetheless, the relevance of sIgA in allergy is still controversial (11), and the role of serum sIgA in natural and induced tolerance to egg in children has not been evaluated before.

In this setting, we designed a research project to study sIgA in natural and induced tolerance to egg proteins. We hypothesized that OIT in egg-allergic children would induce immunological changes involving serum sIgA, mimicking those observed in children who outgrow egg allergy spontaneously.

Methods

Subjects and procedures

Children aged 5–16 years with IgE-mediated egg allergy diagnosis and persistently positive specific IgE (sIgE) (>0.35 kU/l) or skin prick test (SPT) (>3 mm) to ovalbumin (OVA) or ovomucoid (OVM) were recruited. All had strictly avoided egg since diagnosis. A double-blind placebo-controlled food challenge (DBPCFC) to raw egg white was performed at inclusion to assess clinical reactivity/tolerance. Tolerant children were classified as 'transient egg allergic' (TEA). Reactive children

were classified as 'persistent egg allergic' (PEA) and were further divided into a group undergoing oral immunotherapy (OIT) (PEA-OIT) and a group continuing egg avoidance (PEA-EA), based on parent's will. OIT consisted of increasing doses of raw egg white daily over a scheduled induction phase of 16 weeks. Afterwards, one raw egg twice weekly was given as maintenance dose. Desensitization to one raw egg was proven through an open challenge at the end of induction phase. PEA-EA children underwent a second DBPCFC at 9–12 months after inclusion to assess spontaneous tolerance. Two additional groups were included as controls: atopic non-egg-allergic (nut allergic, ANEA) and nonatopic children (NANEA). Both had consumed egg regularly since infancy.

Clinical data (age, gender, comorbidities, OVA/OVM SPT) were recorded at inclusion in all groups. Serum samples were collected at baseline (T0) in all subjects and also 9 months later (T1) in PEA-OIT and PEA-EA. Specific IgE and sIgA in serum were determined by ImmunoCAP (ThermoFisher Scientific, Uppsala, Sweden), being 0.01 kU/l and 0.01 mg_A/l the lower threshold, respectively.

Statistics

Descriptive data were analysed using mean and standard deviation (SD) for normal parameters, whereas median and range

for non-normal parameters. sIgA was compared (a) at baseline versus T1 in PEA-OIT and PEA-EA children – Wilcoxon test – and (b) among PEA-OIT (T0), PEA-OIT (T1), PEA-EA (T0), PEA-EA (T1), TEA, ANEA and NANEA individuals – Kruskal–Wallis test. Linear correlation between sIgE and sIgA was studied through Spearman's correlation coefficient. PASW Statistics 18.0 (IBM, Chicago, IL, USA) was used for analysis. A two-tailed *P* value <0.05 was considered significant.

Results

A total of 94 children were recruited (21 in TEA; 52 in PEA, of whom 28 underwent OIT (PEA-OIT) and 24 continued avoiding egg (PEA-EA); 10 in ANEA; 11 in NANEA). Mean age was 8.2 years (SD: 2.4) and 52% were male. All groups had similar age and gender distribution. All PEA-OIT children tolerated one raw egg after OIT ('desensitized'). Five PEA-EA children developed spontaneous tolerance over follow-up. PEA-OIT children did not differ in baseline clinical/immunological parameters from PEA-EA (Table 1).

Serum OVA sIgA and OVM sIgA did not change significantly over time in PEA-OIT children (*P* > 0.05, Fig. 1A,B). OVA sIgA increased in 19 PEA-OIT children and OVM sIgA in 11. PEA-OIT children in whom OVA sIgA increased did not differ in any baseline clinical or immunological parameter

Table 1 Clinical and immunological characteristics of children recruited and comparative analysis between PEA-OIT and PEA-EA at inclusion

	PEA-OIT (<i>n</i> = 28)	PEA-EA (<i>n</i> = 24)	TEA (<i>n</i> = 21)	ANEA (<i>n</i> = 10)	NANEA (<i>n</i> = 11)	PEA-OIT vs PEA-EA <i>P</i> value
Descriptive data						
Age (years, mean, SD)	8.16 (1.7)	8.4 (3.5)	8 (2.2)	7.7 (2.1)	8.4 (2.5)	0.438
Gender (%male)	39.3%	58.3%	66.7%	60%	63.6%	0.137
Eczema	67.9%	58.3%	23.8%	30.0%	NA	0.477
Multiple food allergies	75.0%	85.7%	42.9%	0%	NA	0.290
Asthma	42.9%	58.3%	57.1%	40.0%	0%	0.266
Moderate asthma*	25%	25%	0%	20%	0%	0.600
Reaction severity at DBPCFC†						0.748
Grade 1	10.7%	16.7%	NA	NA	NA	
Grade 2	17.9%	20.8%	NA	NA	NA	
Grade 3	42.9%	45.8%	NA	NA	NA	
Grade 4	28.6%	16.7%	NA	NA	NA	
Immunological tests T0, median (range)						
Egg white sIgE (kU/l)	9.49 (0.51–46.2)	8.59 (0.69–36.1)	1.07 (0–4)	0	0	0.219
OVA sIgE (kU/l)	2.37 (0–49)	5.22 (1–20)	0.81 (0–2)	0	0	0.291
OVM sIgE (kU/l)	3.13 (0–54)	4.97 (0–41)	0.4 (0–4)	0	0	0.582
Egg white SPT (mm)	8 (4–13)	9.5 (4.5–16)	5.5 (0–10.5)	0	0	0.061
OVA SPT (mm)	8 (5–14.5)	8 (4–13.5)	4.5 (0–14.5)	0	0	0.693
OVM SPT (mm)	8 (0–14.5)	8.5 (3.5–13.5)	4.5 (0–13)	0	0	0.935
OVA sIgA (mg _A /l)	3.05 (0–37.3)	4.09 (0–40.2)	2.8 (0–8.6)	2.9 (0–14.3)	2.7 (0–8.6)	0.078
OVM sIgA (mg _A /l)	2.01 (0–27.7)	2.45 (0–16.3)	1.7 (0–6.5)	2.8 (0–11.9)	1.9 (0–6.1)	0.173

*According to GEMA Spanish Asthma Guideline (16).

†According to Sampson's anaphylaxis grading (17).

ANEA/NANEA, atopic and nonatopic non-egg-allergic; DBPCFC, double-blind placebo-controlled food challenge; NA, not applicable; PEA-EA, persistent egg-allergic children on egg avoidance; PEA-OIT, persistent egg-allergic children under oral immunotherapy; SD, standard deviation; sIgA, specific IgA; sIgE, specific IgE; SPT, skin prick test; T0, time at inclusion; TEA, transient egg allergic.

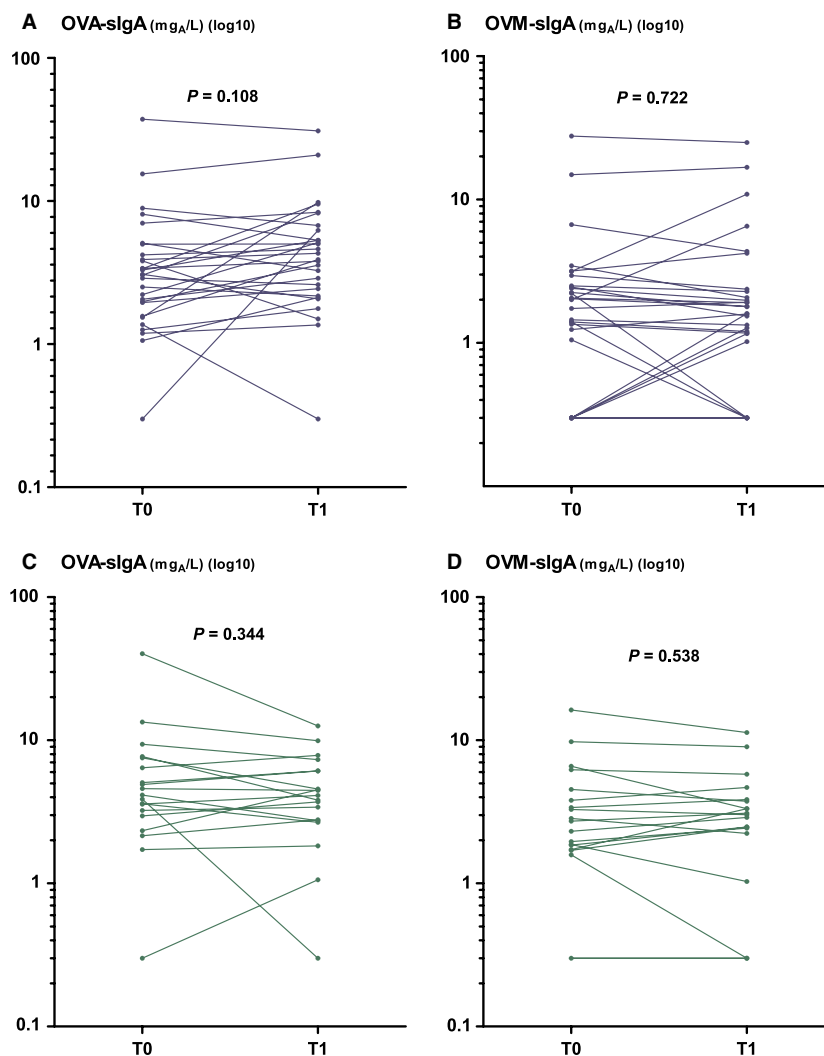


Figure 1 (A–F) Serum specific IgA (sIgA) to ovalbumin (OVA) and ovomucoid (OVM) at baseline (T0) and 9 months later (T1). (A,B) Persistent egg-allergic children desensitized through oral immunotherapy (PEA-OIT); (C,D) persistent egg-allergic children on egg avoidance (PEA-EA) with persistent egg allergy over follow-up; (E,F)

PEA-EA children who develop natural tolerance over follow-up. (G,H) OVA sIgA and OVM sIgA in persistent egg-allergic children at baseline (PEA-OIT T0 and PEA-EA T0), egg-desensitized (PEA-OIT T1), untreated egg-allergic (PEA-EA T1), transient egg-allergic (TEA), atopic (ANEA) and nonatopic (NANEA). Error bars show median values.

from those of PEA-OIT children in whom OVA sIgA did not increase (equivalent results were obtained for OVM sIgA, data not shown, $P > 0.05$). Serum sIgA remained also unchanged in PEA-EA children, both in those 19 who persisted egg allergy over follow-up (Fig. 1C,D) and in those 5 children who developed spontaneous tolerance (Fig. 1E,F). No difference was found in serum sIgA among PEA, TEA, desensitized, ANEA and NANEA individuals. (Table 1, Fig. 1G,H). No linear correlation was found between sIgE and sIgA (Rho Spearman = 0.114 for OVA, $P = 0.198$; Rho Spearman = 0.085 for OVM, $P = 0.339$).

Of note, sIgE decreased over time in PEA-OIT (median OVA sIgE: 2.37 kU/l (T0) vs 1.09 (T1); OVM sIgE: 3.13 vs 1.63, $P < 0.001$) and in all 5 PEA-EA children who developed natural tolerance over follow-up (OVA sIgE: 1.12 vs

0.8; OVM sIgE: 0.98 vs 0.9, $P = 0.062$). Instead, sIgE remained unmodified in those 19 PEA-EA children who persisted allergy at T1 (OVA sIgE: 8.73 kU/l vs 9.14, $P = 0.19$; OVM sIgE: 5.75 vs 5.3, $P = 0.052$).

Discussion

In our study, serum sIgA remained unchanged in egg-allergic children after desensitization. This finding differs from serum sIgA increases detected after peanut SLIT in children (3) and egg OIT in mice (8). No difference in serum sIgA between PEA and TEA children was detected at baseline, and no particular trend was observed in 5 children who developed natural tolerance over follow-up. No difference was found either in serum sIgA among egg-allergic and non-egg-allergic

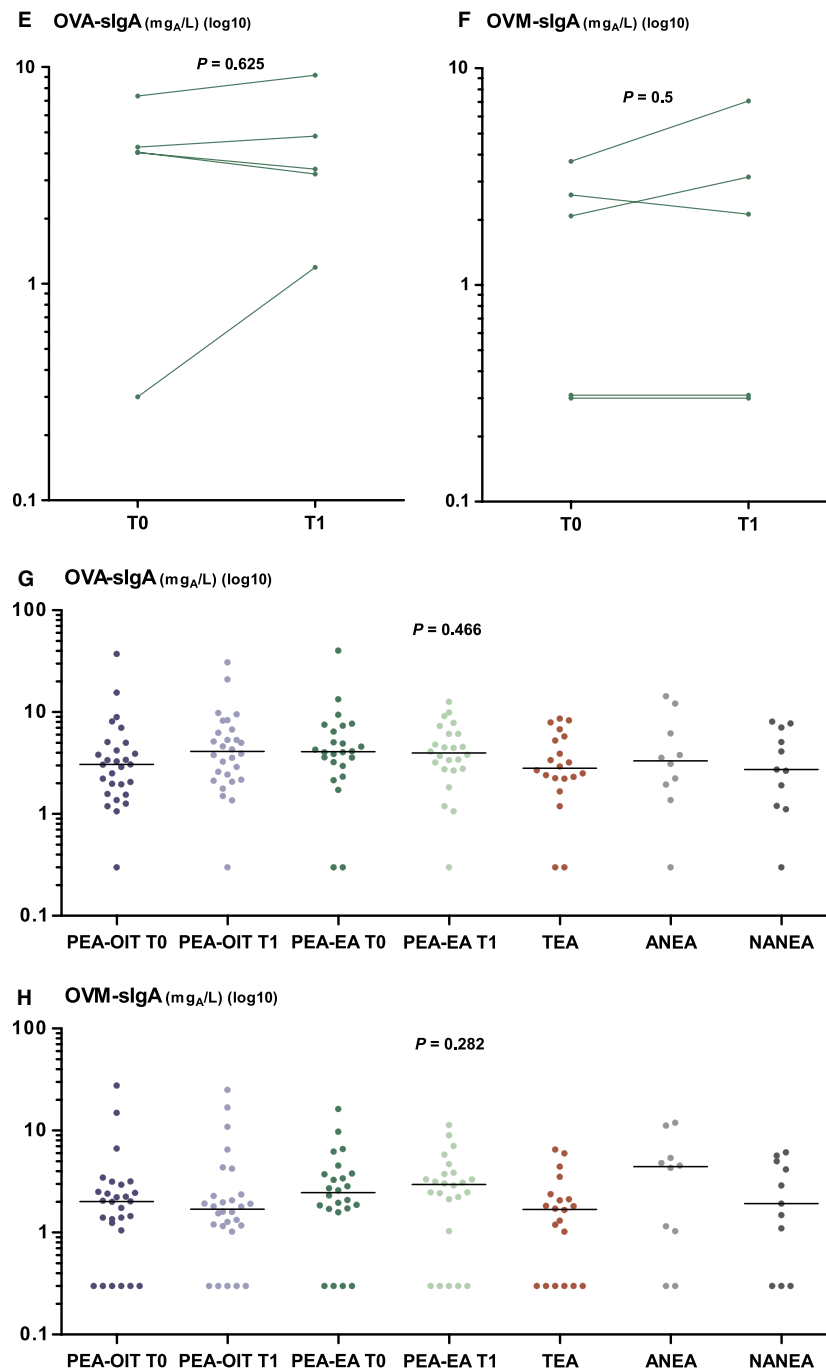


Figure 1 Continued.

children. This contrasts with previous observations of high serum sIgA in beta-lactoglobulin-allergic mice, whereas low in tolerized mice (2). Thus, serum sIgA does not seem to be substantially involved in oral desensitization or in the natural development of tolerance in egg-allergic children. Accordingly, this parameter seems not to be a marker of reactivity or tolerance (natural or induced) to egg allergens.

Similarly, serum OVA/OVM sIgA levels do not seem to be substantially influenced by regular dietary egg exposure/avoidance or by an atopic constitution. In agreement with our findings, previous evidence of a protective role of serum sIgA in food allergy/tolerance in humans is limited and conflicting (11). Previous studies found no differences in serum sIgA among IgE- or non IgE-mediated cow's milk allergic patients

and atopic or non atopic controls (12, 13). In contrast, Savilahti et al. (14) found higher beta-lactoglobulin sIgA at diagnosis in milk-allergic children who outgrew allergy before the third year of age than in children with persistent milk allergy beyond the age of 8. However, sIgA increased over time especially in the persistent group, and the epitope binding pattern of sIgA differed from sIgE (15).

Limitations

Gastrointestinal secretory sIgA was not assessed in this study. Assuming that IgA would be playing its major role in immunity and tolerance at mucosal sites (1, 2), this might explain the lack of evidence of serum sIgA as a relevant tolerance marker or mechanism. Indeed, Leonard et al. observed that a major mechanism of clinical protection induced by OIT in mice was localized in the gastrointestinal tract, although no increase in OVA sIgA in gut lavage could be detected after OIT (8). In view of the above-mentioned results in tolerized and allergic mice (2), serum sIgA levels would not reflect the IgA response at mucosal site. Thus, further research is needed on the role of gastrointestinal secretory sIgA in natural and induced tolerance in children.

We did not determine isotypes of serum sIgA. Although increases in both serum sIgA1 and sIgA2 have been reported after grass pollen SLIT (10), the sIgA response to grass pollen subcutaneous immunotherapy has been reported to be selective for sIgA2 (9). As monomeric IgA1 accounts for 90% of serum IgA (1), differences in sIgA2 might not be detected if only total sIgA is measured.

Conclusions

Serum OVA/OVM sIgA seems not to be associated with induced or natural tolerance to egg allergens. Further research on gastrointestinal secretory sIgA in induced and natural tolerance to food antigens is needed.

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Author contributions

MVO had the original idea for the study, collected and analysed the data and drafted the manuscript. MP, LA and MJ helped with the study design, interpretation of data and critical review of the manuscript. AP and MAMM helped with collection of data and discussion of results. All authors approved the final version of the manuscript.

Conflict of interest

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