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Detection of ovomucoid-specific low-affinity IgE in infants and its relationship to eczema

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Detection of ovomucoid-specific low-affinity IgE in infants and its relationship to eczema

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Abstract

Background: Allergen-specific low-affinity IgE was previously detected in cord blood by a highly sensitive densely carboxylated protein (DCP) chip, but not by ImmunoCAP. Here, we investigated the presence of low-affinity IgE during the early life of infants and observed its relationship with eczema.

Methods: We conducted a birth cohort study, collecting sera at birth and 6 (6M) and 14 months (14M) of age (n=110). We monitored the ovomucoid (OM)- and egg white (EW)-specific-IgE (sIgE) by ImmunoCAP or DCP-chip and analyzed the antigen-affinity of sIgE by binding inhibition assays in the presence or absence of a mild chaotropic agent, diethyl amine (DEA). The low- and high-affinity OM-sIgEs and sensitization risk factors were analyzed by a multivariate logistic analysis.

Results: The OM-sIgE measured by DCP-chip significantly correlated with that measured by ImmunoCAP, but some samples assessed as OM-sIgE-positive by DCP-chip were considered OM-sIgE-negative by ImmunoCAP. Binding inhibition analysis after DEA treatment was performed for participants judged as OM-sIgE-positive by DCP-chip at 14M. The group assessed as negative for OM- and EW-sIgE by ImmunoCAP at 6M and 14M showed a larger binding inhibition

curve shift after DEA treatment than did the group assessed as positive at these times, indicating the presence of low-affinity sIgE antibodies at 14M. The logistic regression analysis found that persistent eczema from 6M to 14M is a significant risk factor for developing high-affinity, but not low-affinity, sIgE.

Conclusions: Human infant peripheral blood contains allergen-specific low-affinity sIgE. Persistent eczema is related to the development of high-affinity, but not low-affinity, IgE.

(250 words; 250 words or less)

Key words

affinity maturation, birth cohort study, eczema, low-affinity IgE, ovomucoid

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Introduction

Immunoglobulin E (IgE) plays key roles in type I hypersensitivity in allergic individuals. Two distinct pathways of IgE class switching have been proposed in mice: a sequential class switching pathway ($\mu \rightarrow \gamma \rightarrow \epsilon$), which can generate high-affinity IgE through somatic hypermutation at IgG1, and a direct class switching pathway ($\mu \rightarrow \epsilon$), which can generate low-affinity IgE (1).

Assessment of the immunological changes that occur during the first year of life is important for understanding the mechanisms of allergen sensitization and allergy development in humans. We previously reported a highly sensitive allergen-microarray on a densely carboxylated protein (DCP)-chip (2, 3), and this method has been validated in successive studies (4-7). By using the DCP-chip

method, we identified a food allergen-specific IgE (sIgE) with low-affinity characteristics in cord blood (CB) that had no cross reactivity to other immunoglobulins (4, 5) and induced an insufficient IgE response in a luciferase-reporting mast cell line, RS-ATL8 cells (5).

Antigens, such as hen's egg proteins, seem to be transferred to the fetus/infant through placenta, breast milk, baby food, or disrupted skin. In the present study, we detected low-affinity ovomucoid (OM)-sIgE in cord blood and during infancy, and we investigated the relationship between low-/high-affinity IgE antibodies and the risk factors for allergic sensitization during infancy, such as feeding, eczema, and solid food consumption.

Methods

Study subjects

We conducted the Gifu Allergy and Immunology Cohort Study (GAICS) (UMIN-CTR registration number: UMIN000002926) (8, 9), the details of which are described elsewhere (8). Written informed consent and CB samples were obtained at the time of birth. To evaluate the extent of maternal blood contamination, CB serum samples with IgA ≥ 1 mg/dL were excluded (10). Physical examination by pediatric allergists and blood sampling at 6 and 14 months were performed, and the participants who had enough samples to assess DCP-chip and ImmunoCAP at all three time points were included in this study (n = 110). Baseline characteristics of the study population were shown in Table 1. Eczema was defined as doctor-diagnosed eczema including atopic dermatitis (AD) and asteatotic eczema. Diagnoses of AD were made by pediatric allergists according to Hanifin's and Rajka's minor criteria (11). Questionnaire-based information was obtained, such as the weekly egg consumption of the mothers, type of feeding (breast-feeding and/or bottle-feeding), and timing of solid food introduction. This study was approved by the Ethics Committees of the Graduate School of Medicine, Gifu University and of the Tokushima University Hospital.

Analysis of sIgE

We analyzed egg white (EW)- and OM-sIgE levels in samples of CB and peripheral blood (PB) using the DCP-chip and ImmunoCAP (Phadia, Uppsala, Sweden) systems. The detection limits of DCP-chip and ImmunoCAP were 20 BUe/mL and 0.1 kU_A/L, respectively. For ImmunoCAP, sIgE-positive samples were defined as those with values of ≥ 0.35 kU_A/L, as suggested by the manufacturer.

Affinity characteristics of OM-sIgE

The affinity characteristics of OM-sIgE in samples taken at 14 months of age were determined on an OM-immobilized DCP-chip through binding inhibition in the presence of a mild chaotropic agent, diethyl amine (DEA), as described previously (5, 12). Briefly, DEA was added to serially buffer-diluted serum at a final concentration of 40 mM, and each sample was applied to an OM-immobilized DCP-chip. After incubation at 37 °C for 60 min followed by washing, the bound antibody was detected by a secondary antibody against human IgE. The resulting fluorescence was plotted against the serum dilution, and the leftward shift of the dose-response curve in the presence of DEA was measured at 50% of the maximum fluorescence.

Statistical analysis

Spearman's rank correlation analysis, Mann-Whitney U-test and Kruskal-Wallis test were performed using GraphPad Prism (GraphPad Software Inc., CA, USA). The ability of the OM-sIgE values measured by DCP-chip to discriminate between levels of OM-sIgE measured by ImmunoCAP as no less than 0.35 kU_A/L and those assessed as less than this amount was evaluated using receiver operating characteristic (ROC) curves in GraphPad Prism. The optimal cutoff point was determined using JMP (SAS Institute Inc. Cary, NC, USA). A *p*-value of <0.05 was considered significant. Univariate and multivariate logistic regression analyses were performed with SPSS software (SPSS, Inc., Chicago, IL, USA).

Results

Comparison of the OM-sIgE levels measured by ImmunoCAP and DCP-chip

We measured the OM-sIgE levels in our samples using both the DCP-chip and ImmunoCAP systems. We then tested the correlation between the OM-sIgE values at 6 and 14 months of age measured by DCP-chip and those measured by ImmunoCAP (Fig. 1A). The values measured by ImmunoCAP and DCP-chip are significantly correlated with one another with a correlation coefficient of 0.6265 (*p* < 0.0001) according to a Spearman's rank correlation test. To determine the cutoff point for predicting which samples should be assessed as sIgE-positive by DCP-chip, a ROC curve analysis was carried out based on the cutoff value for ImmunoCAP (≥ 0.35 kU_A/L) (Fig. 1B). The area under the curve (AUC) of these values is 0.9545 [95% confidence interval (CI): 0.9292–0.9799; *p* < 0.0001], and the optimal cutoff point is 59.8 BUe/mL. Based on the result of a correlation analysis,

OM-sIgE levels of ≥ 60 BUe/mL from DCP-chip, which corresponds to 0.35 kUA/L of the ImmunoCAP system, were defined as positive.

Discrepancy of the OM-sIgE values obtained by DCP-chip and ImmunoCAP

OM-sIgE levels at birth (in CB) and at 6 and 14 months (in PB) as measured by DCP-chip and ImmunoCAP are shown in Figs. 2A and 2B, respectively. All CB samples were negative for OM-sIgE according to the results from the ImmunoCAP, but many of them were assessed as positive by DCP-chip.

When we focused on the results at 14 months of age, we noticed that, among the 110 participants, 45 (40.9%) were assessed as positive for OM-sIgE by DCP-chip, but only 23 participants (20.9%) were considered positive for OM-sIgE by ImmunoCAP. We hypothesized that the discrepancy between the DCP-chip and ImmunoCAP values at 14 months is due to the presence of low-affinity IgE.

Detection of low-affinity sIgE in PB

To confirm whether or not low-affinity sIgE exist in PB as well as in CB, we selected two representative groups among the 45 participants who were positive for OM-sIgE according to DCP-chip; [A] ImmunoCAP-negative group ($n = 9$), composed of those with OM- and EW-sIgE levels from the ImmunoCAP of < 0.35 kUA/L at both 6 and 14 months, and [B] ImmunoCAP-positive group ($n = 10$), composed of those with corresponding levels of ≥ 0.35 kUA/L at both time points.

We analyzed the affinity characteristics of OM-sIgE binding to OM immobilized on the DCP-chip in the presence or absence of DEA. Figs. 3A and 3B show plots of representative samples analyzed by DCP-chip from the ImmunoCAP-positive and -negative groups, respectively. Binding of OM-sIgE on the chip in serially diluted sera in the presence of 40 mM DEA was monitored by fluorescence intensity, which shifted leftward. The magnitudes of the leftward shift at 50% of maximum fluorescence were expressed as $\log_{10}$ values (Fig. 3C). The DEA-induced shift in the ImmunoCAP-negative group was significantly greater than that in the ImmunoCAP-positive group ($p < 0.0001$). This result indicates the presence of low-affinity IgE in the serum, even in 14-month-old children.

The relationship between low- and high-affinity sIgE and sensitization risk factors

Further investigation was performed using the following definitions: low-affinity characteristic indicates OM-sIgE-positive by DCP-chip but OM-sIgE-negative by ImmunoCAP; high-affinity characteristic indicates OM-sIgE-positive by both DCP-chip and ImmunoCAP; and non-sensitized indicates OM-sIgE-negative by both DCP-chip and ImmunoCAP. All participants who were OM-sIgE-positive according to DCP-chip at 6 months were also assessed as OM-sIgE-positive by ImmunoCAP, which indicates the presence of high-affinity IgE characteristics in these subjects.

To investigate the impact of allergen sensitization through the mother's breast milk, the relationship between the mother's weekly egg consumption when the subjects were 6 months of age and the high or low affinity characteristics of the baby's OM-sIgE were studied. In our cohort, only one participant was exclusively fed baby formula; all other participants were fed either only breast milk ($n = 28$) or both breast milk and baby formula ($n = 81$). Among the 110-paired samples, we obtained information about the mother's egg consumption during the baby's first six months from 99 participants. The averages of weekly egg consumption ($\pm$ standard deviation, n = numbers of participants) in non-sensitized, low-affinity characteristic, and high-affinity characteristic groups at 6 months were 3.8 eggs (± 2.2 , $n = 59$), 4.3 eggs (± 2.0 , $n = 22$), and 4.9 eggs (± 2.3 , $n = 18$), respectively, which are not significantly different from one another ($p = 0.2021$ by Kruskal-Wallis test).

The dual-allergen exposure hypothesis was recently proposed (13); it holds that tolerance occurs as a result of oral exposure to food, and allergic sensitization results from cutaneous exposure. Thus, we divided the participants into four groups based on the presence of eczema at 6 and 14 months, and the affinity patterns at 14 months were investigated (Table 2): [i] no-eczema: no eczema at either 6 or 14 months; [ii] late-onset: no eczema at 6 months, with eczema at 14 months; [iii] (early onset and) persistent: having eczema both at 6 and 14 months; [iv] (early onset and) transient: having eczema at 6 months and no eczema at 14 months. The percentages of high-affinity characteristics in the no-eczema, late-onset, transient, and persistent groups were 6.9%, 40.0%, 40.0%, and 47.8%, respectively, which suggest that persistent eczema during infancy might be related to the development of high-affinity IgE. The no-eczema group showed the highest prevalence of low-affinity IgE and the lowest prevalence of high-affinity IgE characteristics.

A multivariate logistic regression analysis, adjusted for feeding exclusively breast milk, mother's egg consumption (>1 egg daily), sex, and low birth weight or

premature infant, was performed to predict the risk for developing low- or high-affinity OM-sIgE (Table 2). The no-eczema group was set as a reference. The adjusted odds ratios (aOR) of late-onset, transient, and persistent groups for developing OM-specific low- or high-affinity characteristic IgE are shown in Table 1. All other variables, including feeding exclusively breast milk, mother's daily egg consumption, sex, and low birth weight or premature infant, were not statistically significant. These results indicate that persistent eczema is related to the development of high-affinity OM-sIgE during early life.

Discussion

We successfully measured OM-sIgE in the early life of 110 Japanese children from the GAICS birth cohort (8, 9) using DCP-chip, which is a newly developed highly sensitive antigen-specific immunoglobulin assay system (2-4). Although we previously reported the existence of low-affinity IgE in human CB (5), no evidence had been published showing the existence of low-affinity IgE in PB after birth. In this study, we report the existence of low-affinity OM-sIgE during the first year of life. We also found a relationship between the affinity maturation of allergen-sIgE antibodies and risk factors for food sensitization, such as eczema.

DEA treatment analysis with DCP-chip showed the existence of low-affinity OM-sIgE. Low-affinity antibodies were more susceptible to the DEA treatment than high-affinity antibodies (5, 12). As we described in the supplemental materials in our previous publication, we already ruled out the cross reactivity of other immunoglobulins to the DCP-chip(5). We also previously demonstrated that the low-affinity food allergen-sIgE in CB showed insufficient IgE-mediated human FcεRI cross-linking to induce the activation of RS-ATL8 cells, a luciferase-reporting mast cell line (5). Then, we concluded that the DEA-induced shift indicates the presence of low-affinity IgE in the serum, even in 14-month-old children.

Whether sensitization in utero occurs or not has been under debate. Previous studies reported that the IgE detected in CB was transferred from the mother (14, 15) and that sensitization patterns of maternal and fetal IgE are highly compatible (16). Additionally, there is a transplacental passage of maternal IgE in the form of anti-IgE IgG/IgE immune complexes through the Fc receptor (FcRn) (17). However, recent studies demonstrated differential sensitization profiles between blood from mothers and that from infants (4, 18). Importantly, fetuses seem to be capable of generating IgE antibodies (19, 20). Therefore, IgE antibodies likely begin to develop in utero.

The fact that many of the participants in our cohort had low-affinity OM-sIgE at birth supports the possibility of maternal–fetal antigen transfer followed by sensitization in utero. Rath et al. demonstrated that IgG is bidirectionally transported by FcRn in human placental endothelial cells in the same manner as IgG transport in the intestinal epithelium (21). It seems possible that antigens are transferred to the fetus by antigen–IgG complexes (5). Interestingly, this transferred antigen is only capable of inducing low-affinity IgE antibodies. The amount of antigen transferred in utero may be limited in this system, so in addition to the immaturity of the fetal immune system, this may be a reason that only low-affinity IgE was detectable in CB.

Breast milk is considered to be a major source of allergen delivery during early infancy. OM is detected in human breast milk either as non-degraded proteins alone (22) or as protein-IgA immune complexes (22, 23). Many participants showed a marked decrease in OM-sIgE levels at 6 months according to DCP-chip, which means that, in many infants, the hen's egg antigen in breast milk may not be a triggering antigen even for low-affinity IgE antibodies. There was no significant difference in the mothers' weekly egg consumption among the groups, indicating that the antigen delivery through mother's milk may not be the major factor for OM-sIgE affinity maturation.

Another possible sensitization pathway is through disrupted skin (13, 24). Our present data indicate that early onset persistent eczema might be a risk factor for developing high-affinity IgE characteristics. Indeed, several papers have shown that hen's egg allergens are detected in house dust (25, 26). These data suggest the possible involvement of skin barrier dysfunction in the affinity maturation of IgE. Notably, antigens exposed on the skin surface may have a relatively higher possibility of being natural and undigested antigens than those from breast milk, which can stimulate antigen-presenting cells more efficiently.

The role of allergen-specific low-affinity IgE remains enigmatic at present. Xiong et al. reported that high-affinity IgE causes anaphylaxis, whereas low-affinity IgE does not (1). They postulated that low-affinity IgE may compete with high-affinity IgE for binding to FcεR and weaken the response of mast cells. Moreover, experiments in human basophils showed that re-sensitization with low-affinity IgE clones results in lower sensitivity and lower maximal activation levels than re-sensitization with high-affinity IgE clones (27). In contrast, several studies showed the contribution of low-affinity IgE antibodies to basophil activation (28-30). The low-affinity OM-sIgE antibodies detected in our study seem to be different from high-affinity antibodies in terms of their developmental pathways and their role in infants.

In conclusion, we discovered that low-affinity OM-sIgE is present in PB during infancy, in addition to being present in CB. Furthermore, we investigated the relationship between OM-sIgE affinity characteristics and the risk factors for food sensitization in early infancy, and we found that the development of high-affinity IgE, but not of low-affinity IgE, is related to eczema. Additional studies are needed to elucidate the roles of low-affinity IgE and of their affinity maturation to high-affinity after birth.

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Conflict of interest

The authors declare no conflicts of interest.

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Figure legends

Fig. 1. Relationship between the OM-sIgE levels measured by DCP-chip and those measured by ImmunoCAP.

(A) Correlation between the OM-sIgE levels measured by DCP-chip and those measured by ImmunoCAP for samples from 6 months or 14 months. (B) A ROC curve analysis was performed to obtain the optimal cutoff DCP-chip values for predicting which samples should be assessed as OM-sIgE-positive, corresponding to ≥ 0.35 kU_A/L by ImmunoCAP.

Fig. 2. Age-dependent changes in OM-sIgE levels.

The OM-sIgE levels were analyzed by the DCP-chip or ImmunoCAP systems. Scatter plots of the OM-sIgE levels measured by DCP-chip (A) or ImmunoCAP (B)

of paired samples (n = 110) are shown. The dotted line represents the detection limit of DCP-chip (A) or ImmunoCAP (B).

Fig. 3. Effect of 40 mM DEA on the binding of serially diluted sera from PB samples from 14-month-old children. The binding curves of OM-sIgE to the chip in the presence (grey dashed lines) or absence (black solid lines) of DEA were obtained by serial dilution of PB samples from 14-month-old children in the ImmunoCAP-positive group (A) or ImmunoCAP-negative group (B). (C) Comparison between the two groups of the amount of $\log_{10}$ shift in the curves due to the addition of DEA. The line indicates the geometric means of each group. The *p*-value was calculated by using a Mann-Whitney U-test.

Table 1 Baseline characteristics of the study population

Total numbers of study participants	n = 110	
Sex (Male : Female)	50 : 60	
Gestational age	278.3 days	(± 6.7)
	(equivalent to 39 weeks and 5.3 days)	
Premature infants	n = 2	
Birth weight	3103 g	(± 374.2)
Low birth weight	n = 3	(2390-2498 g)
Allergic disease at 6 months of age		
AD	n = 28	(25.5 %)
eczema	n = 33	(30.0 %)
RW	n = 0	(0.0 %)
FA	n = 0	(0.0 %)
Allergic disease at 14 months of age		
AD	n = 24	(21.8 %)
eczema	n = 28	(25.5 %)
RW	n = 7	(6.4 %)
FA	n = 18	(16.4 %)
Questionnaire based information at 6 months of age		
Type of feeding		
Exclusively breast-fed	n = 28	(25.5 %)
Mixed		
Mainly breast-fed	n = 57	(51.8 %)
Mainly bottle-fed	n = 24	(21.8 %)
Exclusively bottle-fed	n = 1	(0.9 %)
Mothre's egg consumption	4.2 eggs/week	(± 2.3) (n = 105)

Table 2 Disease course of eczema and its relationship of low- and high-affinity IgE

Name of the groups	Eczema		Numbers of participants	OM-sIgE (DCP-chip at 14 months)											
	6M	14M		DCP-positive		Low-affinity characteristic					High-affinity characteristic				
				n	%	n	%	aOR*	95% CI	<i>p</i>	n	%	aOR*	95% CI	<i>p</i>
No-eczema group	-	-	72	23	31.9%	18	25.0%				5	6.9%			
Late-onset group	-	+	5	2	40.0%	0	0.0%	-	-	-	2	40.0%	1.98	0.29–13.75	0.49
Transient group	+	-	10	5	50.0%	1	10.0%	0.43	0.05–3.76	0.44	4	40.0%	2.84	0.55–14.79	0.22
Persistent group	+	+	23	15	65.2%	4	17.4%	0.95	0.27–3.37	0.94	11	47.8%	9.05	2.45–33.52	0.01

aOR: adjusted odds ratio; 95% CI: 95% confidential interval, *p*: *p*-value; n: number of participants; %: percentage of participants

*Adjusted for feeding exclusively breast milk, daily egg consumption >1, sex, and low-birth-weight or premature infant

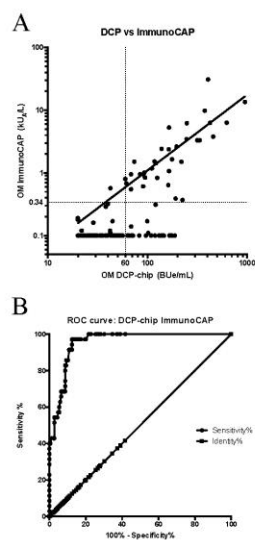


Figure 1

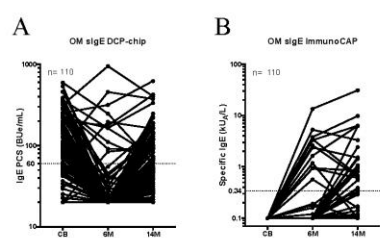


Figure 2

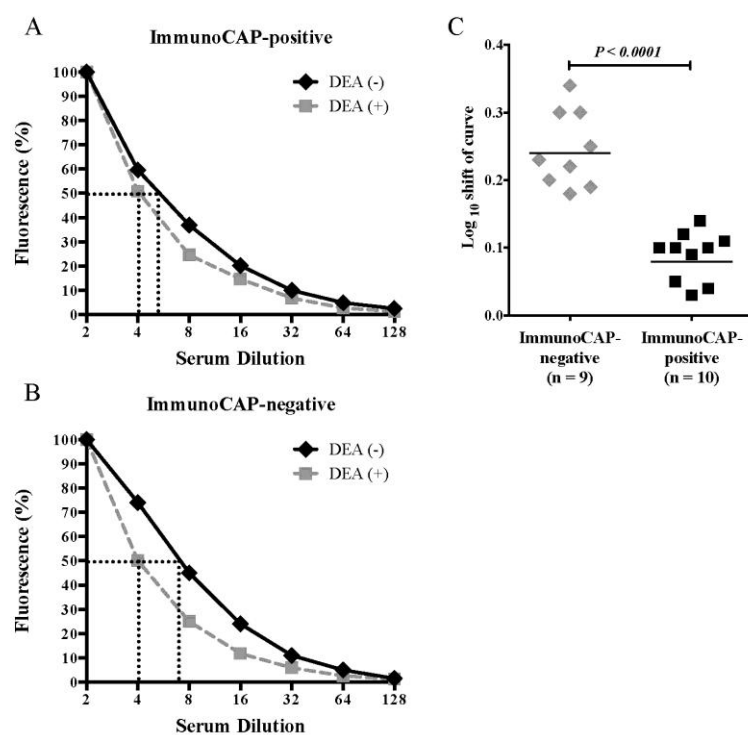


Figure 3