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Original Article

Activated steady status and distinctive FcεRI-mediated responsiveness in basophils of atopic dermatitis

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AD, atopic dermatitis; HCs, healthy controls; FcεRI, high-affinity IgE receptor; BAT, basophil activation test; MFI, mean fluorescence intensity; EASI, eczema area and severity index; TARC, thymus and activation-regulated chemokine; LDH, lactate dehydrogenase; CD203c, ectonucleotide pyrophosphatase (E-NPP3)

ABSTRACT

Background: Although basophils are considered to play an important role for maintenance of type 2 inflammation in atopic dermatitis (AD), studies on basophils in AD patients are limited. Some studies have reported the activation status, including CD203c and CD63, of peripheral blood basophils in AD patients.

Methods: We examined the features of circulating basophils in AD patients, assessed cell surface marker expressions and total serum IgE, and compared basophil responsiveness to stimulation between AD patients and healthy controls (HCs). In addition, the correlations among AD severity, laboratory factors, and features of basophils were examined. Blood samples from 38 AD patients and 21 HCs were analyzed. Basophil response markers CD203c and CD63, and expression of surface-bound IgE and FcεRI on basophils were measured. CD203c and CD63 expressions induced by stimulation with anti-IgE and anti-FcεRI antibodies were measured. Clinical/laboratory factors including total serum IgE were examined for correlations with these basophil parameters.

Results: Baseline CD203c and CD63 expression on basophils were significantly higher in AD patients compared with HCs. The CD203c/CD63 response ratio to anti-FcεRI stimulation was higher than that to anti-IgE stimulation in AD patients, but not HCs. FcεRI expression on basophils was higher in AD patients than in HCs, although surface-bound IgE on basophils was equivalent. Total serum IgE had negative correlations with surface-bound IgE and CD63 responsiveness to anti-IgE stimulation.

Conclusions: Basophils were spontaneously activated under steady-state conditions in AD patients and responsiveness to anti-IgE stimulation was lower than in HCs. Despite high serum IgE and high basophil FcεRI expression, surface-bound IgE on basophils remained relatively low. Basophils might be suppressed or exhausted regarding FcεRI signaling via IgE in severe AD.

Introduction

Atopic dermatitis (AD) is a disease characterized by lesions involving eczema with pruritus, which are repeatedly exacerbated and ameliorated.[1] Immunologically, AD is characterized by overexpression of T helper 2 (Th2) cytokines including interleukin (IL)-4 and IL-13.

Basophils, which have AD-associated features including secretion of Th2 cytokines and histamine release after activation, are the least populated granulocyte in the human body. Therefore, studies on the mechanisms of basophil functions are limited.[2] However, some reports have described that basophils play an important role for maintenance of type 2 inflammation in AD.[3]

In AD model mice, basophils and group 2 innate lymphoid cells (ILC2) were the first cells to infiltrate the skin lesions.[4] Basophil-derived IL-4 was necessary for the promotion of ILC2-mediated inflammation in these model mice.[4] Elimination of basophils from the skin lesions caused reductions in the numbers of infiltrating eosinophils and neutrophils in mouse models.[5] Basophils were also suggested to play an important role in the development of IgE-mediated chronic allergic inflammation as an initiator rather than an effector.[5]

In contrast, studies on basophils in AD patients are limited. Basophils were found in the skin lesions in more than half of AD patients.[6] Regarding basophil response markers, two compartments have been identified: CD203c compartment and CD63

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compartment.[7] CD203c has higher sensitivity and elicits a greater antigen-triggered basophil response than CD63, although CD63 has higher specificity.[8] Upregulation of CD63, but not CD203c, on basophils reflects histamine release more accurately.[9,10] Basal CD63 expression on basophils without stimulation was similar in AD patients and healthy controls (HCs).[11] In most AD patients, CD203c expression on basophils without stimulation was similar to that in healthy controls (HCs); however there were some variations, with the basophils in certain AD patients showing high levels of CD203c expression.[6] Therefore, studies focusing on basophils in AD patients, including basophils under stimulation, are necessary.

Histamine released by basophils is an important inflammatory mediator.[12] However, histamine release by basophils in AD patients with and without stimulation remains controversial. Without any stimulation, basophils in AD patients showed high spontaneous histamine release compared with HC basophils.[13] Some reports documented that histamine release by basophils under anti-IgE stimulation was increased in patients with severe AD.[12] In contrast, other reports described that histamine release was at a similar level to that in HCs[14] or decreased.[15]

The high-affinity IgE receptor (FcεRI) is expressed on mast cells and basophils, and cross-linkage of FcεRI by antigens and specific IgE induces cell activation.[16] Basophils in AD patients expressed more FcεRI than those in HCs and there was a positive correlation between FcεRI and total serum IgE.[17] It has been proposed that FcεRI is controlled by total serum IgE.[18,19] However, the relationship between FcεRI and IgE expression on peripheral blood basophils in AD patients is not completely understood.

High total serum IgE concentration in AD patients was shown to have a positive correlation with some disease severity scores.[20–22] Thus, increased total serum IgE is observed in AD patients depending on the disease severity. However, it remains unclear whether this increase in serum IgE is directly involved in the disease exacerbation and pathogenesis of AD. Elevated serum IgE in AD patients was considered to cause activation of basophils in peripheral blood.[23] Furthermore, some reports mentioned that high serum IgE caused low expression of CD63,[24] while other reports mentioned that high serum IgE caused high expression of CD203c on basophils.[25]

Based on these backgrounds, we focused on the status of peripheral blood basophils in AD patients using CD203c and CD63 as response markers. The relationship between AD severity and basophil responsiveness was examined. To clarify the basophil characteristics in AD patients, surface-bound IgE and FcεRI were also examined and these surface expressions were compared with disease severity and serum IgE. In this study, we found some unique functional and conditional characteristics of basophil in AD patients.

Methods

Study population

Patients with AD who came to the Dermatological Department of Kobe University Hospital and agreed to participate in the study were enrolled. AD was diagnosed in accordance with the criteria in the Guidelines for Atopic Dermatitis.[26] Disease activity in AD patients was assessed by EASI score. Moderate to severe AD patients were mainly enrolled in the study and their median EASI score was 18.5 (Table 1). Patients who had been treated with dupilumab, omalizumab, systemic corticosteroids, or immunosuppressants including cyclosporin and those who had cancer were excluded from the study. HCs without current or previous symptoms of AD who voluntarily agreed to participate in the study were enrolled. Among the HCs, those with allergic disease (allergic rhinitis, asthma, food allergy, allergic conjunctivitis, or urticaria) were excluded from the study. Blood samples from HCs were used for flow cytometric analysis. All participants provided verbal and written informed consent for inclusion in the study. The study protocol was approved by the Institutional Review Board of Kobe University (No. B190182).

Table 1
Clinical characteristics of AD patients and HCs.

HCs (n = 21)			Normal range
Age, y		29 (26–46)	
Sex	Male	7 (33.3%)	
	Female	14 (66.7%)	
Laboratory factors	Total serum IgE (IU/mL)	56.2 (6.7–493)	<295
AD patients (n = 38)			
Age, y		45.5 (20–63)	
Sex	Male	25 (65.8%)	
	Female	13 (34.2%)	
Disease duration, y		36.0 (6–57)	
EASI score		18.5 (1.0–54.3)	
Laboratory factors	TARC (pg/mL)	2010 (310–28604)	<450
	LDH (U/L)	219.5 (150–486)	124–222
	Total serum IgE (IU/mL)	6336.3 (140.8–85805.8)	<295
	Eosinophils (cells/μL)	597.5 (15.5–3388.5)	30–350
	Basophils (cells/μL)	61.05 (27.0–208.8)	0–190
Complications	Allergic rhinitis	17 (45%)	
	Asthma	16 (42.1%)	
	Food allergy	11 (28.95%)	
	Allergic conjunctivitis	7 (18%)	
	Urticaria	6 (15.79%)	
Treatments	Topical steroids	38 (100%)	
	H1 antihistamines	28 (73.68%)	
	Topical tacrolimus	19 (50%)	
	Ultraviolet B therapy	1 (2.63%)	

EASI, eczema area and severity index; TARC, thymus and activation-regulated chemokine; LDH, lactate dehydrogenase; MFI, mean fluorescence intensity.
Data are shown as median (range) for age, disease duration, EASI score, and laboratory factors and as n (%) for sex, complications, and treatments.

Basophil activation test

Flow cytometric analysis based on the basophil activation test (BAT) was performed with Allergenicity Kit (Beckman Coulter, Brea, CA) to measure CD203c.[27] We added some reagents to measure additional parameters of CD63, surface-bound IgE, and FcεRI expressions. About 2 mL of whole blood samples for the BAT were collected from AD patients and HCs into blood collection tubes with ethylenediaminetetraacetic acid (EDTA). The BAT was performed within 24 h of blood sampling.

Fifty microliters of whole blood with EDTA, 10 μL of cocktail staining reagent and 50 μL of activation buffer were mixed in fluorescence-activated cell sorting (FACS) tubes. The cocktail staining contains fluorescein isothiocyanate (FITC)-conjugated anti-human CRTH2 antibody (clone: BM16), phycoerythrin (PE)-conjugated anti-human CD203c antibody (clone: 97A6), and phycoerythrin-cyanine 7 (PC7)-conjugated anti-human CD3 antibody (clone: UCHT1). Next, 10 μL of PBS as a negative control, 10 μL of anti-IgE antibody as a positive control, 0.5 μL of CD63, 0.6 μL of surface-bound IgE, or 1.25 μL of anti-FcεRI antibody was mixed into individual FACS tubes. The FACS tubes were incubated at 37 °C for 15 min. Biotinylated anti-FcεRI antibody was coupled with 1 μL of APC-Streptavidin as a second-step reagent at 4 °C for 30 min. Erythrocytes were depleted by adding fixative and lysis buffer for 10 min, followed by centrifugation at 200×*g* for 5 min. After removal

of the supernatant, the cells were washed with 1500 μL of PBS, centrifuged at 200×*g* for 5 min, and fixed with 300 μL of 0.1% formaldehyde. Basophil samples were measured by flow cytometry (FACS Verse; BD Biosciences, San Jose, CA). The flow cytometry data were analyzed with FlowJo software (BD Biosciences, Franklin Lakes, NJ). More detail about basophil detection, reagents, and flow cytometrical gating technique are described in Supplementary Figure 1.

Reagents preparation

Phosphate-buffered saline (PBS) was used as a negative control. Anti-IgE antibody (clone: E124-2-8D) from the Allergenicity Kit was used as a positive control (1 μg/mL) to stimulate basophils. PacificBlue-conjugated anti-human CD63 antibody (clone: H5C6; BioLegend, San Diego, CA) (0.9 μg/mL) was used to measure CD63 expression. VioBlue-conjugated anti-human IgE antibody (clone: MB10-5C4; Miltenyi Biotec, Bergisch Gladbach, Germany) (0.5 μg/mL) was used to measure surface-bound IgE. Biotinylated anti-human FcεRI antibody (clone: CRA1; Bio-Academia, Osaka, Japan) (11.2 μg/mL) was used to measure FcεRI expression. FcεRI expression corresponded with total FcεRI expression because the anti-FcεRI antibody used binds to the stalk region of the protein and does not inhibit IgE-binding.[28] Anti-FcεRI antibody was also used as a stimulant for basophils. Basophil responsiveness to anti-FcεRI stimulation was measured.[29] APC-Streptavidin (BD Biosciences, Franklin Lakes, NJ) (1.8 mg/mL) was used as a second-step reagent for the anti-FcεRI antibody. Mite allergen extract was kindly provided by Torii Pharmaceutical Co. Ltd. (Tokyo, Japan). Freeze-dried allergen contained Der f1 (6 μg/mL) and Der f2 (3.6 μg/mL). We stimulated basophils with mite allergen (0.5, 5, and 50 ng/mL).

Basophil detection

We detected and characterized basophils using forward scatter (FSC), side scatter (SSC), and fluorochromes (FITC-conjugated anti-human CRTH2 antibody, PE-conjugated anti-human CD203c antibody, PC7-conjugated anti-human CD3 antibody, PacificBlue-conjugated anti-human CD63 antibody, VioBlue-conjugated anti-human IgE antibody, biotinylated anti-human FcεRI antibody). Histograms were created for FSC and SSC to eliminate red blood cell debris and select the lymphocytes and monocytes population. CD3-positive T-lymphocytes were eliminated by PC7. Basophils were selected as the CRTH2-positive/CD203c-positive/CD3-negative population. Basophil activation was detected as the CD203c/CD63-high population. For flow cytometry, all plots of the target parameter were measured and recorded, and the mean value was calculated and defined as the mean fluorescence intensity (MFI). Based on the MFI, CD203c and CD63 expression under the condition with no stimulation (PBS) were defined as 'baseline MFI', those under the condition with anti-IgE antibody stimulation were defined as 'anti-IgE stimulation MFI', and those under the condition with anti-FcεRI antibody stimulation were defined as 'anti-FcεRI stimulation MFI'.[30] To calculate the responsiveness of basophils, we divided stimulation MFI by baseline MFI and presented it as the 'response ratio'. The gating technique is shown in the Supplementary Figure 1.

Statistics

Data were analyzed and plotted with GraphPad Prism8 software (GraphPad Software Inc., La Jolla, CA). Statistical analyses were performed using the nonparametric Mann–Whitney U test and the parametric unpaired *t*-test. Significance was considered for values of $P < .05$. To determine the correlations among data, Spearman rank correlation coefficient analysis was performed.

Results

Study population

The characteristics and laboratory data for the AD patients and HCs are shown (Table 1). The number of AD patients was 38 and the median age was 45.5 (20–63) years. The male-to-female ratio was 25 (65.8%) to 13 (34.2%). Disease duration was 36.0 (6–57) years. Median EASI score was 18.5 (1.0–54.3). Median total serum IgE was 6336.3 IU/mL. Complications with other allergic diseases were allergic rhinitis in 17 (45.0%), asthma in 16 (42.1%), food allergy in 11 (29.0%), allergic conjunctivitis in 7 (18%), and urticaria in 6 (15.8%). Thirty-eight AD patients received topical steroids (100%), 28 received H1 antihistamines (73.68%), 19 received topical tacrolimus (50%), and one received ultraviolet B therapy (2.63%).

The number of HCs was 21 and the median age was 29.0 (26–46) years. The male-to-female ratio was 7 (33.3%) to 14 (66.7%). The median total serum IgE level was 56.2 IU/mL.

Analysis of clinical and laboratory factors in AD patients

Clinical factors, disease severity reflected by EASI scores,[31] and laboratory factors including thymus and activation-regulated chemokine (TARC), lactate dehydrogenase (LDH), and total serum IgE were analyzed in AD patients.[32] Among the AD patients, moderate positive correlations were observed between EASI score and TARC ($r_s = .50$) and between EASI score and LDH ($r_s = .67$) (Fig. 1A, B). Although not statistically significant, there was a mild positive correlation between EASI score and total serum IgE ($r_s = .31$) (Fig. 1C).

Analysis of basophil response markers including CD203c and CD63

To determine the baseline basophil status in AD patients, we analyzed basophil activation status using the basophil response markers of CD203c and CD63 in both AD patients and HCs. Unlike a previous report,[6] AD patients had a higher baseline CD203c ($P < .001$) and a lower CD203c response ratio with anti-IgE stimulation ($P < .001$) than HCs (Fig. 2A, B). Baseline CD63 in AD patients was higher than that in HCs ($P < .001$) (Fig. 2C). CD63 response ratio with anti-IgE stimulation was lower in AD patients than in HCs ($P < .001$) (Fig. 2D). Thus, CD203c with no stimulation and with anti-IgE stimulation on AD basophils showed a similar expression pattern to CD63. These data suggest that AD basophils were activated spontaneously with no stimulation and exhibited low responsiveness to anti-IgE stimulation.

Correlations between CD203c/CD63 response ratio and clinical/laboratory factors

Because basophils in AD patients exhibited characteristic responses to anti-IgE antibody stimulation, we examined the correlations between these expression patterns and clinical/laboratory factors, including EASI score, TARC, LDH, and total serum IgE. There were no correlations between baseline CD203c and factors (Supplementary Fig. 2), baseline CD63 and factors (Supplementary Fig. 3), and CD203c response ratio and factors (Supplementary Fig. 4). CD63 response ratio had moderate negative correlations with EASI score ($r_s = -.38$) and TARC ($r_s = -.38$) (Fig. 3A, B). Although statistically not significant, there was a trend toward a negative correlation between CD63 response ratio and LDH ($r_s = -.28$) (Fig. 3C). CD63 response ratio had a moderate negative correlation with total serum IgE ($r_s = -.37$) (Fig. 3D).

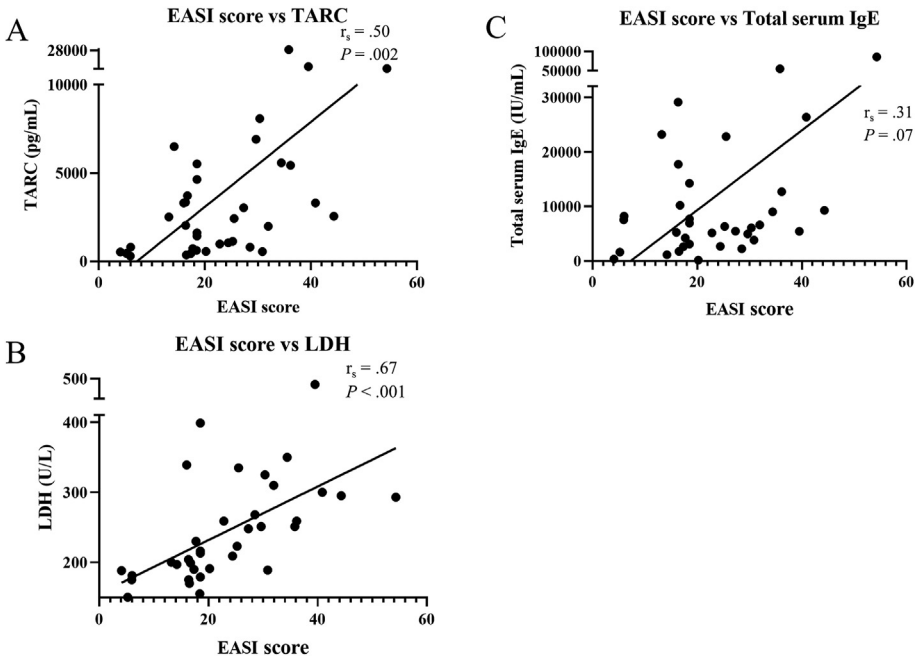

Fig. 1. Correlations between EASI score and laboratory factors in AD patients. **(A)** TARC. **(B)** LDH. **(C)** Total serum IgE. Statistical analyses were performed using Spearman's rank correlation coefficient.

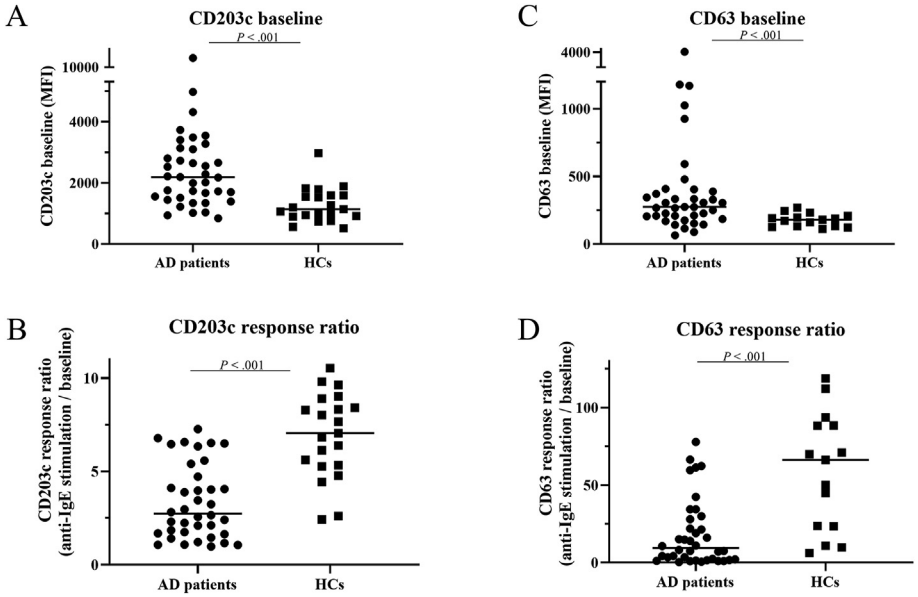

Fig. 2. Comparison of basophil responsiveness without stimulation and with anti-IgE antibody stimulation between AD patients and HCs. **(A)** Baseline CD203c. **(B)** CD203c response ratio. **(C)** Baseline CD63. **(D)** CD63 response ratio. Line indicates median. Statistical analyses were performed using the Mann–Whitney U-test.

Analysis of FcεRI expression and surface-bound IgE on basophils

Because correlations were observed between the clinical factors, especially total serum IgE, and responsiveness of basophils, we further examined IgE-related surface markers, including FcεRI expression and IgE expression on basophils. AD patients exhibited higher FcεRI expression on basophils than HCs ($P < .001$) (Fig. 4A). However, FcεRI had no correlations with clinical/laboratory factors of EASI score, TARC, and LDH (Supplementary Fig. 5). There was a negligible negative trend between FcεRI and serum IgE ($r_s = -.23$) (Supplementary Fig. 5D). In contrast, despite high serum IgE and higher FcεRI expression on basophils in AD patients compared with HCs, there was no significant difference in surface-bound IgE between AD patient and HCs ($P = .52$) (Fig. 4B). Moreover, surface-bound IgE had moderate negative correlations with EASI score ($r_s = -.35$) and TARC ($r_s = -.35$) (Fig. 5A, B). Although statistically not significant, there was a trend toward a negative correlation between surface-bound IgE and LDH ($r_s = -.24$) (Fig. 5C). Surface-

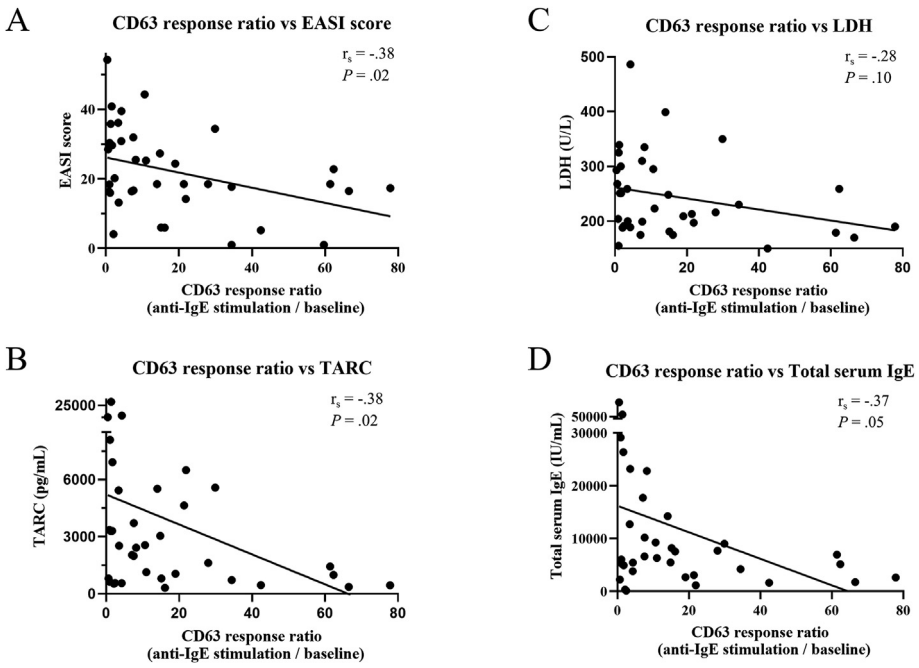

Fig. 3. Correlations between CD63 response ratio and clinical/laboratory factors in AD patients. **(A)** EASI score. **(B)** TARC. **(C)** LDH. **(D)** Total serum IgE. Statistical analyses were performed using Spearman's rank correlation coefficient.

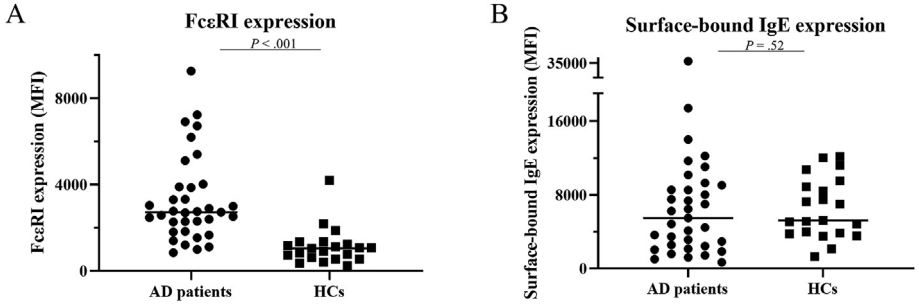

Fig. 4. Comparison of FcεRI expression and surface-bound IgE expression between AD patients and HCs. **(A)** FcεRI expression. **(B)** Surface-bound IgE. Line indicates median. Statistical analyses were performed using the Mann–Whitney U-test.

bound IgE had a strong negative correlation with total serum IgE ($r_S = -.70$) (Fig. 5D). In short, basophils in AD patients, especially in severe AD patients, showed the paradoxical status that surface-bound IgE was kept low despite high FcεRI expression on basophils and high total serum IgE (Fig. 5A, 4A, 1C).

Comparison of anti-FcεRI stimulation versus anti-IgE stimulation

Because we observed a paradoxical surface-bound IgE status in AD basophils, we refocused on the differences in two other stimulations of FcεRI. Specifically, we compared the response ratios after anti-FcεRI stimulation and anti-IgE stimulation in AD patients and HCs. In HCs, the CD203c response ratio for anti-FcεRI/baseline was lower than anti-IgE/baseline ($P < .001$) (Fig. 6B). This observation was also found in our previous report.[29] In contrast, the CD203c response ratio in AD patients was higher for anti-FcεRI/baseline than for anti-IgE/baseline ($P = .02$) (Fig. 6A). Thus, the responsiveness of basophils to anti-FcεRI stimulation and anti-IgE stimulation was opposite between AD patients and HCs. From another point of view, the responsiveness of basophils in AD patients was maintained for anti-FcεRI stimulation, but reduced for anti-IgE stimulation.

Comparison of mite allergen stimulation

We examined the responsiveness of basophils to real allergens rather than antibodies using mite allergen, which includes Der f1/f2 as a representative allergen to which most AD patients are sensitized. We compared the CD203c/CD63 response ratio in AD basophils following mite stimulation. The CD203c and CD63 response ratios both increased in an allergen-concentration-dependent manner (Fig. 7A, B).

Discussion

In this study, we examined the IgE-related surface markers on peripheral blood basophils and the responsiveness of basophils stimulated with FcεRI. Regarding surface markers, there was no significant difference in surface-bound IgE between AD patients and HCs (Fig. 4B). Obviously, total serum IgE was very high in AD patients (Table 1). However, a negative correlation between total serum IgE and surface-bound IgE in AD patients was observed (Fig. 5D). Patients with severe AD tended to have high levels of total serum IgE and low levels of surface-bound IgE on basophils (Fig. 1C, 5A), despite higher expression of FcεRI in AD patients compared

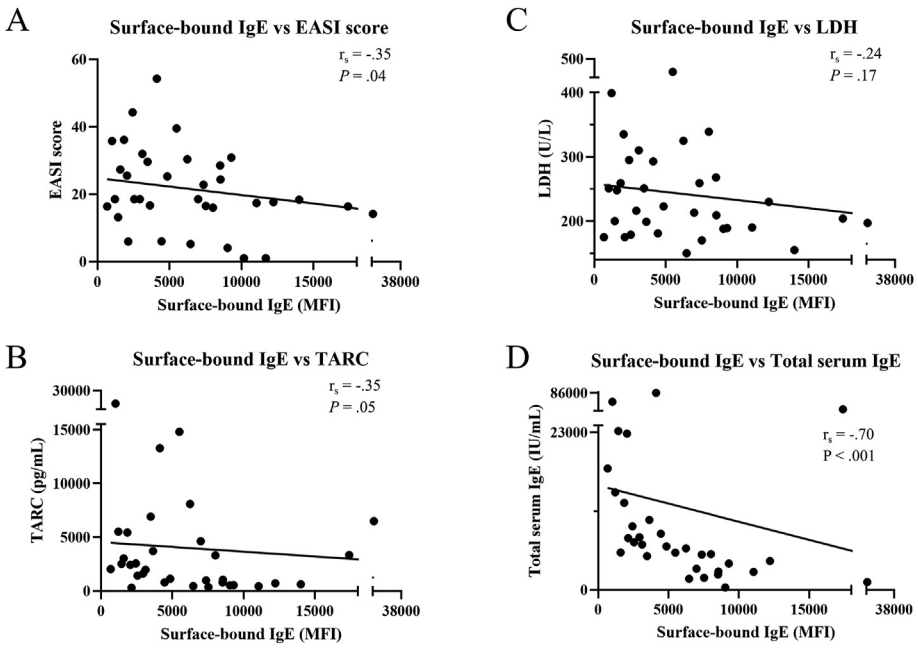

Fig. 5. Correlations between surface-bound IgE and clinical/laboratory factors in AD patients. **(A)** EASI score. **(B)** TARC. **(C)** LDH. **(D)** Total serum IgE. Statistical analyses were performed using Spearman's rank correlation coefficient.

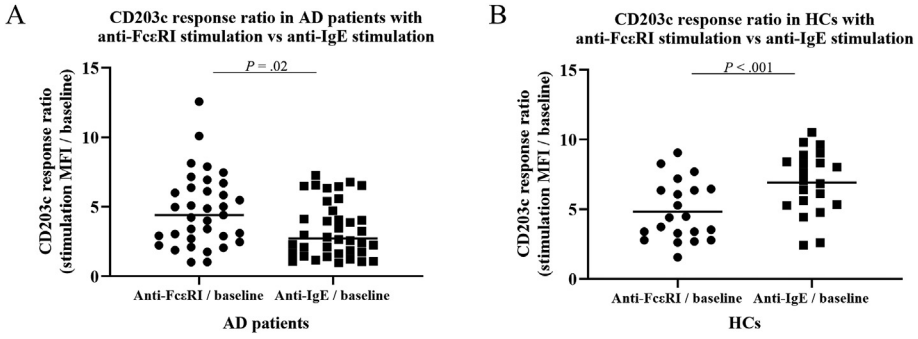

Fig. 6. Comparison of CD203c response ratios with anti-FcεRI stimulation or anti-IgE stimulation. **(A)** AD patients. Line indicates median. Statistical analyses were performed using the Mann–Whitney U-test. **(B)** HCs. Line indicates mean. Statistical analyses were performed using the unpaired *t*-test.

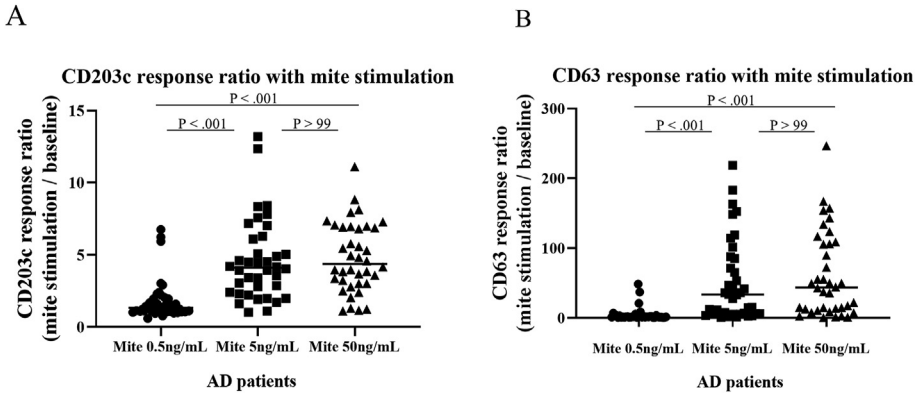

Fig. 7. Comparison of CD203c/CD63 response ratios with mite allergen stimulation in AD patients. **(A)** CD203c response ratio. **(B)** CD63 response ratio. Line indicates median. Statistical analyses were performed using Kruskal–Wallis test followed by Dunn's multiple comparison.

with HCs (Fig. 4A). These results suggested that activation of Th2 inflammation would lead to the production of total serum IgE and FcεRI on basophils in AD patients. However, the antibodies or receptors might have been functionally incomplete, thus preventing effective binding, or some other substances may have blocked binding. Whatever the cause, the increased serum IgE did not bind efficiently to basophils in this study. In contrast, Yanase *et al.* reported that a high concentration of IgE caused histamine release, polarization, and CD203 upregulation in human basophils without stimulation *in vitro*.[25] Although they did not examine whether the high concentration of IgE actually bound to FcεRI, they concluded that a high concentration of IgE modified the function of basophils. Although a high concentration of total serum IgE may have not bound to FcεRI on basophils in this study, increased serum IgE may have adjusted basophil activation indirectly, leading to the formation of a vicious circle between high serum IgE and basophils.

In addition, expression of FcεRI was higher in AD patients than in HCs (Fig. 4A). FcεRI on basophils was reported to be controlled by total serum IgE.[18,19] However, our study revealed that elevated serum IgE had no correlation with baseline CD203c, baseline CD63, and FcεRI on basophils, respectively (Supplementary Fig. 2D, 3D, 5D).

This study revealed that basophils in AD patients exhibited low responsiveness, including CD203c and CD63, to anti-IgE stimulation (Fig. 2B, D). There were moderate negative correlations between CD63 responsiveness with anti-IgE stimulation and EASI score or TARC, suggesting that the responsiveness of basophils to anti-IgE antibody stimulation decreased as AD became more severe (Fig. 3A, B). It is possible that binding of IgE on basophils affected the responsiveness of basophils to anti-IgE antibody stimulation. Therefore, we examined the binding status of IgE on basophils in the comparison between AD patients and HCs, and the correlation between surface-bound IgE and AD severity. However, surface-bound IgE was equivalent between AD patients and HCs, indicating that the low responsiveness to anti-IgE stimulation when comparing AD patients in general and HCs cannot be explained by surface IgE binding status alone.

Our findings also demonstrated that AD patients had higher baseline CD203c and CD63 levels than HCs (Fig. 2A, C). These findings may indicate that AD basophils were spontaneously activated to release histamine and inflammatory mediators without FcεRI stimulation. Because upregulation of CD63 reflects histamine release,[33] it is possible that AD basophils may already be mildly exhausted in the steady state. Since the basophils in AD patients had already been activated and exhausted without stimulation, we assumed that it was more difficult to activate these cells by anti-IgE stimulation compared with those in HCs even if the binding sites for anti-IgE antibodies were equivalent. In contrast, there were negative correlations between EASI score and CD63 response ratio (Fig. 3A) and between EASI score and surface-bound IgE (Fig. 5A). These findings suggested that the reduced surface-bound IgE expression on basophils observed in severe AD patients can explain the decreased responsiveness for anti-IgE stimulation in severe AD patients.

We also used an anti-FcεRI antibody as a stimulus. The anti-FcεRI antibody binds to the stalk region of FcεRI and does not inhibit IgE binding.[28] This antibody can bind receptors directly, unlike anti-IgE antibody which binds to receptors indirectly via IgE. In this study, the CD203c response ratio of anti-FcεRI/baseline was lower than that of anti-IgE/baseline in HCs, consistent with a previous report.[29] This means that the anti-IgE antibody under our experimental conditions could increase the HC basophil responsiveness more efficiently than the anti-FcεRI antibody. We consider that this result was caused by the different binding sites of the two antibodies. Also, the expression of FcεRI on basophils was higher in AD patients than in HCs (Fig. 4A). Based solely on this expression level, the responsiveness of basophils to anti-FcεRI in AD patients is presumed to be higher than that in HCs. However, in fact, AD basophils exhibited equivalent responsiveness to anti-FcεRI to HC basophils (Fig. 6A, B). This low responsiveness to anti-FcεRI in AD basophils may also be associated with the exhaustion of basophils, similar to the phenomenon of the low responsiveness to anti-IgE stimulation in AD patients.

Unexpectedly, our results showed that responsiveness to mite allergen stimulation was dose-dependent (Fig. 7). However, mite allergen can stimulate basophils via IgE-dependent as well as IgE-independent pathways. MRGPRX2, as a receptor mediating IgE-independent activation, was shown to be expressed on human basophils,[34] and the mite allergen Der p1 and hexapeptides derived from Der p1 activated MRGPRX2.[35,36] Thus, although the IgE-dependent pathway of anti-IgE stimulation showed low responsiveness in AD basophils in this study, mite allergen might have activated basophils via an IgE-independent pathway. Furthermore, we assumed that only FcεRI pathways via IgE might have been suppressed, because the response to anti-FcεRI stimulation was higher than that to anti-IgE stimulation. Overall, these results suggested that various factors interacted with each other and caused basophil activation, involving different binding sites for each antibody, differences between stimulants, and basophil exhaustion due to spontaneous activation in the steady state.

There are some limitations to our small-scale study according to the number of participants and sex adjustment. The HC participants were significantly younger than the AD patients, which might have affected the results. In addition, AD can be classified as intrinsic or extrinsic type,[37] based on a threshold concentration of total serum IgE (serum IgE < 150 IU/mL, intrinsic type; serum IgE > 150 IU/mL, extrinsic type).[38] Our study only enrolled two patients with intrinsic AD, and we could not therefore analyze enough number of intrinsic AD. It is possible that the inclusion of more patients with intrinsic AD might affect the results, and IgE-caused basophil activation might be limited to extrinsic AD.

In conclusion, we addressed the following hypothesis: type 2 inflammation in AD stimulates B cells and B cells secrete high concentration of IgE.[39,40] However, the elevated IgE in AD did not bind efficiently to circulating basophils. AD basophils were spontaneously activated and exhibited low responsiveness against anti-IgE stimulation. Some AD basophils, especially severe AD basophils, behaved like low responders for anti-IgE stimulation, but not for anti-FcεRI stimulation. Further studies are required to determine the physiological meaning for this distinctive basophil status in AD.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.alit.2021.01.005.

Conflict of interest

AF has received speaking honoraria from Sanofi. The rest of the authors have no conflict of interest.

Authors' contributions

KW and AF designed the study. SI and AF, wrote the original draft. SI, KW, YO, AF, CN edited the manuscript. SI, YO, MM contributed to data collection. SI performed the statistical analysis and interpretation of the results. All authors read and approved the final manuscript.

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