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

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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. OBJECTIVE: To examine the features of circulating basophils in AD patients by assessing and comparing cell surface marker expressions, total serum IgE, and basophil responsiveness to stimulation between AD patients and healthy controls (HCs). In addition, to examine the correlations among AD severity, laboratory factors, and features of basophils. METHODS: 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: Basophils in AD patients were activated spontaneously without stimulation and exhibited low responsiveness to anti-IgE antibody stimulation. Responsiveness to anti-FcεRI stimulation was higher than anti-IgE stimulation, contrary to 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: Our findings illustrate the characteristic basophil status in AD. Despite high serum IgE and high basophil FcεRI expression, surface-bound IgE on basophils remained relatively low and basophils were spontaneously activated under steady-state conditions.

1 | 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. Therefore, many AD patients have a predisposition toward allergy, i.e., a condition that readily produces allergen-specific IgE antibodies that are activated and produced by Th2 cytokines.[2]

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.[3]However, some reports have described that basophils play an important role for maintenance of type 2 inflammation in AD.[4]

In AD model mice, basophils and group 2 innate lymphoid cells (ILC2) were the first cells to infiltrate the skin lesions.[5]Basophil-derived IL-4 was necessary for the promotion of ILC2-mediated inflammation in these model mice.[5] The TSLP-dendritic cell-T cell pathway caused basophils to release IL-4 and induce Th2 polarization in mice.[6] Elimination of basophils from the skin lesions caused reductions in the numbers of infiltrating eosinophils and neutrophils in mouse models.[7]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.[7]

In contrast, studies on basophils in AD patients are limited. Basophils were found in the skin lesions in more than half of AD patients.[8] Regarding basophil response markers, two compartments have been identified: CD203c compartment and CD63 compartment.[9] Upregulation of CD63, but not CD203c, on basophils reflects histamine release.[10, 11]In most AD patients, CD203c expression on basophils without stimulation was similar to that in healthy controls (HCs); however there was some variation, with the basophils in certain AD patients showing high levels of CD203c expression.[8] Basal CD63 expression on basophils without stimulation was similar between AD patients and HCs.[12] However, studies focusing on the properties of circulating basophils in AD patients, including basophils under anti-IgE and anti-FcεRI stimulation, are limited.

Histamine released by basophils is an important inflammatory mediator.[13] 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.[14,15,16] Some reports documented that histamine release by basophils under anti-IgE stimulation was increased in patients with severe AD.[13,15] In contrast, other reports described that histamine release was at a similar level to that in HCs[16] or decreased.[17]

The high-affinity IgE receptor (FcεRI) is expressed on mast cells and basophils, and cross-linkage of FcεRI by allergens and specific IgE induces cell activation.[14] Expression of FcεRI on mast cells was upregulated by IL-4.[18] Preincubation with anti-FcεRI antibody caused histamine degranulation in response to various stimulations in human cultured basophils.[16]Basophils in AD patients expressed more FcεRI than those in HCs and there was a positive correlation between FcεRI and total serum IgE.[19] In addition, omalizumab, a humanized anti-IgE monoclonal antibody, caused downregulation of FcεRI expression and responsiveness to anti-IgE stimulation.[20] It has been proposed that FcεRI is controlled by total serum IgE.[20,21] 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 scoring of atopic dermatitis (SCORAD).[22] Another study showed a correlation between total serum IgE concentration and disease severity in severe AD patients.[23] Total serum IgE level and peripheral eosinophils were correlated with the eczema area and severity index (EASI) score.[24] 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. In fact, a systematic review documented that the recommendations for omalizumab use in clinical practice for AD are limited.[25] In contrast, elevated serum IgE in AD patients was considered to cause activation of basophils in peripheral blood.[26]Furthermore, some reports mentioned that high serum IgE caused low expression of CD63, a response marker on basophils,[27]while other reports mentioned that high serum IgE caused high expression of CD203c on basophils.[28]

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 status in AD patients.

2 | MATERIALS AND METHODS

2.1 | 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.[29, 30] 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 S1). Patients who had been treated with dupilumab, oral 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.

2.2 | Basophil activation test

Flow cytometric analysis based on the basophil activation test (BAT) was performed with an Allergenicity Kit (Beckman Coulter, Brea, CA) to measure CD203c.[31] We added some reagents to measure additional parameters of CD63, surface-bound IgE, and FcεRI expressions. 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 hours of blood sampling.

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-IgE antibody (clone: MB10-5C4; Miltenyi Biotec, Bergisch Gladbach, Germany) (0.5 μg/mL) was used to measure surface-bound IgE. Biotinylated anti-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.[32] Anti-FcεRI antibody was also used as a stimulant for basophils. Basophil responsiveness to anti-FcεRI stimulation was measured.[33] APC-Streptavidin (BD Biosciences, Franklin Lakes, NJ) (1.8 mg/mL) was used as a second-step reagent for the anti-FcεRI antibody.

Fifty milliliters of whole blood with EDTA, 10 μL of mixed staining reagent containing CRTH2-FITC, CD203c-PE, and CD3-PC7, and 50 μL of activation buffer were mixed in FACS tubes. 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 for 15 minutes. Biotinylated anti-FcεRI antibody was coupled with 1 μL of APC-Streptavidin as a second-step reagent at 4 for 30 minutes. Erythrocytes were depleted by adding fixative and lysis buffer for 10 minutes, followed by centrifugation at $200\times g$ for 5 minutes. After removal of the supernatant, the cells were washed with 1500 μL of PBS, centrifuged at $200\times g$ for 5 minutes, 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).

For basophil detection and characterization, we employed forward scatter (FSC), side scatter (SSC), and fluorochromes (CRTH2-FITC, CD203c-PE, anti-FcεRI antibody-APC, CD63 and surface-bound IgE-Pacific blue, and CD3-PC7). Histograms were created for FSC and SSC to eliminate red blood cell debris and select the total leucocyte 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. Mean fluorescence intensity (MFI) was measured. 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'.[34] 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 supplemental material (Figure S1).

2.3 | 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.

3 | RESULTS

3.1 | Study population

The characteristics and laboratory data for the AD patients and HCs are shown in Table S1. 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%). 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

3.2 | Analysis of c linical and laboratory factors in AD patients

Clinical factors, disease severity reflected by EASI scores,[35] and laboratory factors including thymus and activation-regulated chemokine (TARC), lactate dehydrogenase (LDH), and total serum IgE were analyzed in AD patients.[36] Among the AD patients, moderate positive correlations were observed between EASI score and TARC ($r_s = .50$) and between EASI score and LDH ($r_s = .67$) (Figure 1 A, B). Although not statistically significant, there was a mild positive correlation between EASI score and total serum IgE ($r_s = .31$) (Figure 1 C).

3.3 | 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 CD203c and CD63 in both AD patients and HCs. Unlike a previous report,[8] AD patients had a higher baseline CD203c ($P < .001$) and a lower CD203c response ratio with anti-IgE stimulation ($P < .001$) than HCs (Figure 2 A, B). Baseline CD63 in AD patients was higher than that in HCs ($P< .001$) (Figure 2 C). CD63 response ratio with anti-IgE stimulation was lower in AD patients than in HCs ($P < .001$) (Figure 2 D). 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.

3.4 | 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 (Figure S2), baseline CD63 and factors (Figure S3), and CD203c response ratio and factors (Figure S4). CD63 response ratio had moderate negative correlations with EASI score (r_s = -.38) and TARC (r_s = -.38) (Figure 3 A, B). Although statistically not significant, there was a trend toward a negative correlation between CD63 response ratio and LDH (r_s = -.28) (Figure 3 C). CD63 response ratio had a moderate negative correlation with total serum IgE (r_s = -.37) (Figure 3 D).

3.5 | Αναλψσις οφ ΦcεPI εξπρεσσιον ανδ συρφαcε-βουνδ IγE ον βασοπηιλς

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 ($P < .001$) (Figure 4 A). However, FcεRI had no correlations with clinical/laboratory factors of EASI score, TARC, and LDH (Figure S5). There was a negligible negative trend between FcεRI and serum IgE (r_s = -.23) (Figure S5 D). 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 ($P = .52$) (Figure 3 B). Moreover, surface-bound IgE had moderate negative correlations with EASI score (r_s = -.35) and TARC (r_s = -.35) (Figure 5 A, B). Although statistically not significant, there was a trend toward a negative correlation between surface-bound IgE and LDH (r_s = -.24) (Figure 5 C). Surface-bound IgE had a strong negative correlation with total serum IgE (r_s = -.70) (Figure 5 D). 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 (Figure 1 C, Table S1).

3.6 | ὂμπαρισον οφ αντι-ΦςεPI στιμυλατιον ερσυς αντι-IγE στιμυλατιον

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 ($P < .001$) (Figure 6 B). This observation was also found in our previous report.[33] In contrast, the CD203c response ratio in AD patients was higher for anti-FcεRI/baseline than for anti-IgE/baseline ($P = .02$) (Figure 6 A). 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.

4 | DISCUSSION

In this study, we examined the IgE-related surface markers on peripheral blood basophils and the responsiveness of basophils stimulated with FcεRI by comparing AD patients and HCs. We particularly focused on the relationships between clinical/laboratory factors such as disease severity and serum IgE in AD patients and these parameters. Regarding surface markers, there was no significant difference in surface-bound IgE between AD patients and HCs (Figure 4 B). Obviously, total serum IgE was very high in AD patients (Table S1). However, a negative correlation between total serum IgE and surface-bound IgE in AD patients was observed (Figure 5 D). Severe AD patients tended to have a high level of total serum IgE and a low level of surface-bound IgE (Figures 1 C, 5 A). Our findings also demonstrated that AD patients had higher baseline CD203c and CD63 than HCs (Figure 2 A, C). These findings may indicate that AD basophils were spontaneously activated to release histamine and inflammatory mediators, possibly including IL-4, without FcεRI stimulation. Because basophils stimulate B cells to synthesize IgE in an IL-4-dependent manner,[37]this basophil-derived IL-4 can contribute to the increase in serum IgE in AD patients. However, the increased serum IgE did not bind well to basophils for some reason. In contrast, Yanase *et al* .[28] reported that a high concentration of IgE caused histamine release, polarization, and CD203 upregulation in human basophils without stimulation *in vitro* . 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 the present study, increased serum IgE may have adjusted basophil activation indirectly, leading to the formation of a vicious circle between high serum IgE and basophils.

On the contrary, expression of FcεRI was higher in AD patients than in HCs (Figure 4 A). FcεRI on basophils was reported to be controlled by total serum IgE.[20,21] However, our study revealed that elevated serum IgE had no correlation with baseline CD203c, baseline CD63, and FcεRI on basophils, respectively (Figure S2 D, S3 D, S5 D). Another interesting observation was that IgE poorly bound to AD basophils despite elevated FcεRI expression. Previous reports documented that human basophils expressed IL-4 receptors[38] and survival of mice basophils were enhanced by IL-4.[39]Also, FcεRI expression on mast cells was upregulated by IL-4.[18] Because secretion of IL-4 is increased in typical AD patients, FcεRI on basophils may have the possibility of being controlled by IL-4.[40] Moreover, because AD patients had higher baseline CD203c and CD63 than HCs in our study, activated basophil-derived IL-4 may contribute to the high expression of FcεRI on AD basophils in an autocrine fashion. It is possible that the increased FcεRI expression and activated steady status on AD basophils observed in this study was increased by AD-related cytokines such as IL-4.

This study revealed that basophils in AD patients exhibited low responsiveness, including CD203c and CD63, to anti-IgE stimulation (Figure 2 B, 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 (Figure 3 A, 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 and HCs cannot be explained by surface IgE binding status alone. AD basophils were activated spontaneously with no stimulation (Figure 2 A, C). Because upregulation of CD63 reflects histamine release,[41] 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 (Figure 3 A) and between EASI score and surface-bound IgE (Figure 5 A). 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 AD patients in general.

Finally, we used an anti-FcεRI antibody as another stimulus to examine the responsiveness of basophils. The CD203c response ratio of anti-FcεRI/baseline was lower than that of anti-IgE/baseline in HCs, consistent with a previous report.[33] This means that the anti-IgE antibody under our experimental conditions could increase the HC basophil responsiveness more efficiently than the anti-FcεRI antibody. The anti-FcεRI antibody binds to the stalk region of FcεRI and does not inhibit IgE binding.[32] We consider that this result was caused by the different binding sites of the two antibodies. The expression of FcεRI on basophils was higher in AD patients than in HCs (Figure 4 A). 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, AD basophils exhibited equivalent responsiveness to anti-FcεRI to HC basophils (Figure 6 A, B). This relatively weak responsiveness to anti-FcεRI in AD basophils may also be associated with the exhaustion of basophils in the steady state, similar to the phenomenon of the low responsiveness to anti-IgE stimulation in AD patients.

There are some limitations to our small-scale study according to the number of participants and sex adjustment. We could not examine the association between basophils and cytokines including IL-4, and histamine released from basophils. Furthermore, the study focused on circulating basophils and did not examine basophils in AD skin lesions.

In conclusion, we addressed the following hypothesis: type 2 inflammation in AD stimulates B cells and B cells secrete high concentration of IgE.[40,42] 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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Author Contributions:

SI performed, analyzed, interpreted experiments, and wrote the manuscript. KW supervised experiments and edited the manuscript. MM performed experiments. YO performed experiments, contributed to technical help, and edited the manuscript. AF designed, analyzed, supervised experiments, and edited the manuscript. CN edited the manuscript. All authors revised and approved the final version.

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Figures

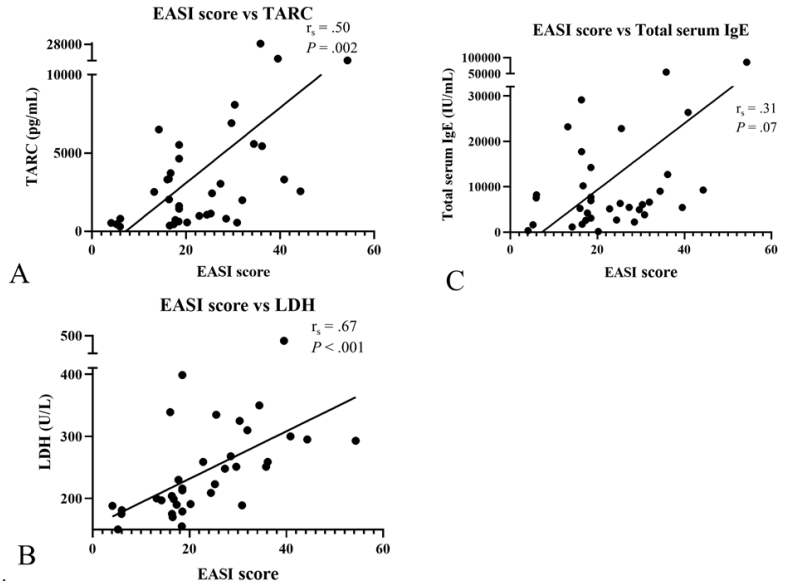
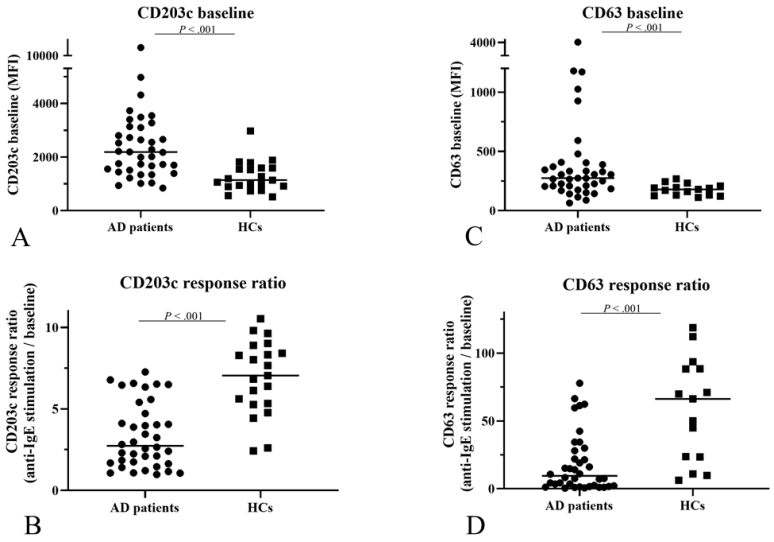


FIGURE 1.



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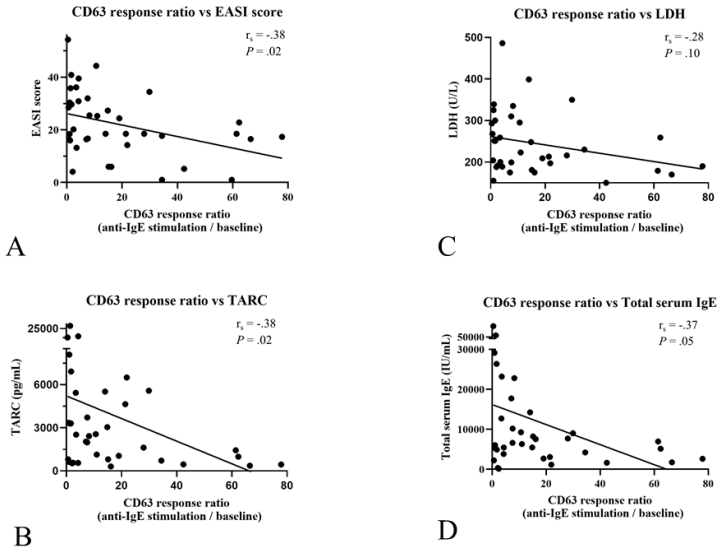

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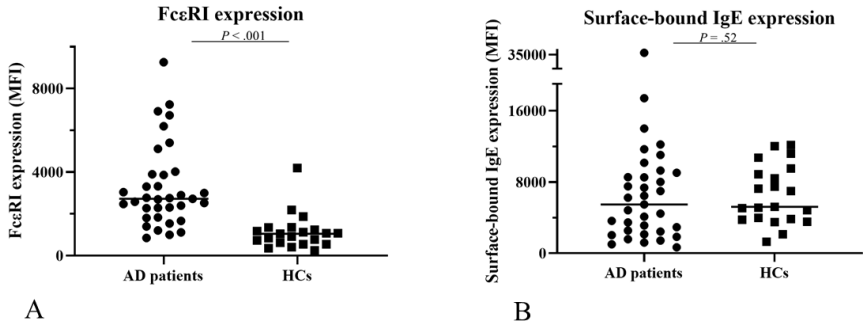

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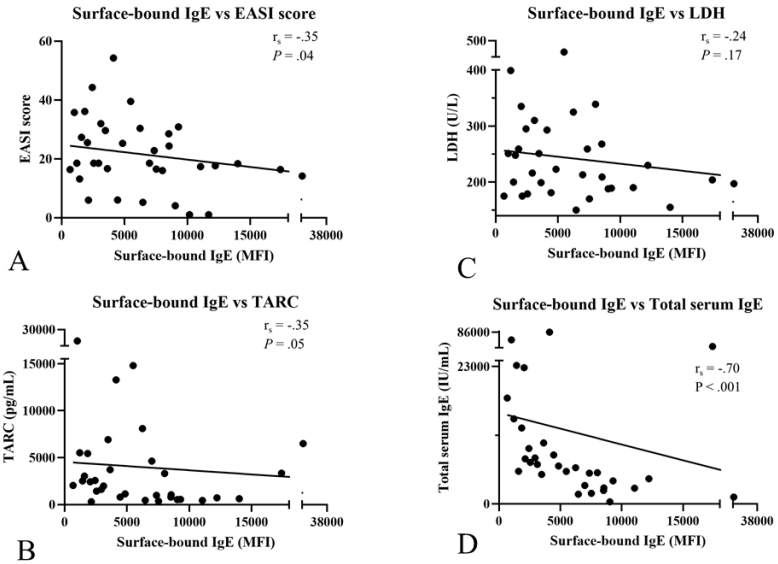

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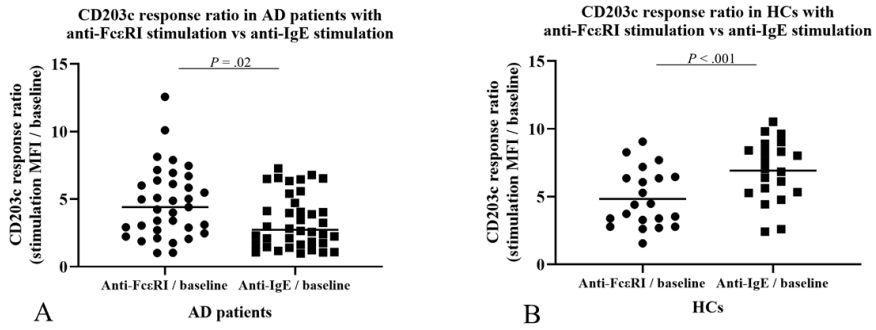


FIGURE 6.

Figure legends

FIGURE 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.

FIGURE 2. Comparison of basophil responsiveness without stimulation (A, C) and with anti-IgE antibody stimulation (B, D) 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.

FIGURE 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.

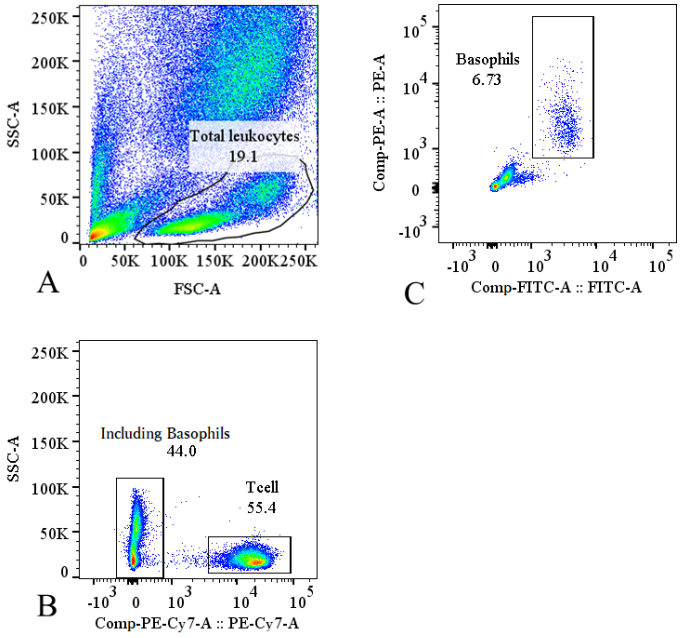
FIGURE 4. Comparison of FcεRI expression and surface-bound IgE expression between AD patients and HCs. (A) FcεRI expression. (B) Surface-bound IgE. Line indicates median. Statistical analyses were performed using the Mann–Whitney U-test.

FIGURE 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.

FIGURE 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.

Supplemental Figures



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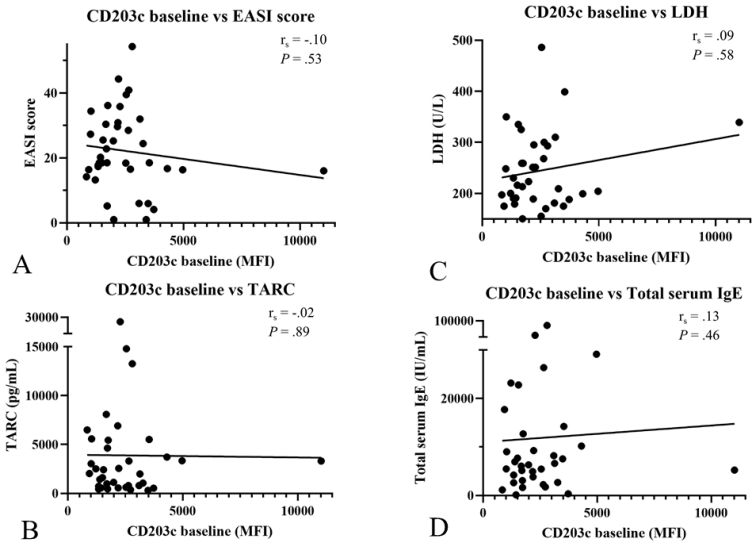

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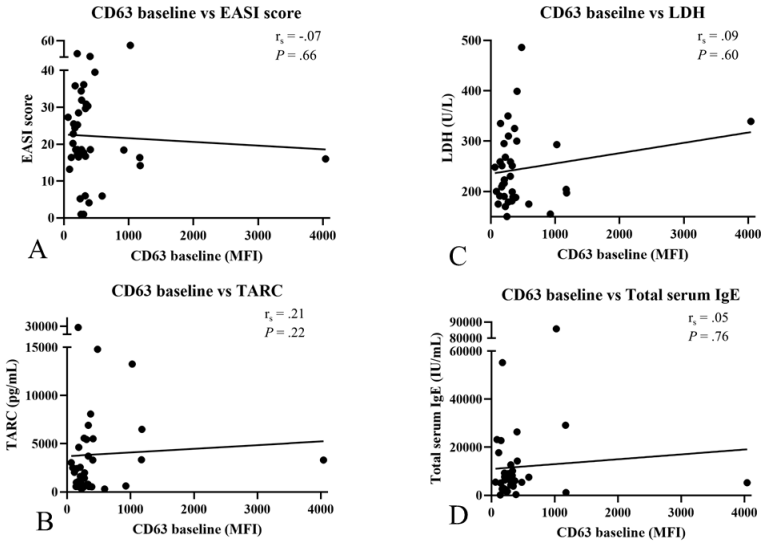

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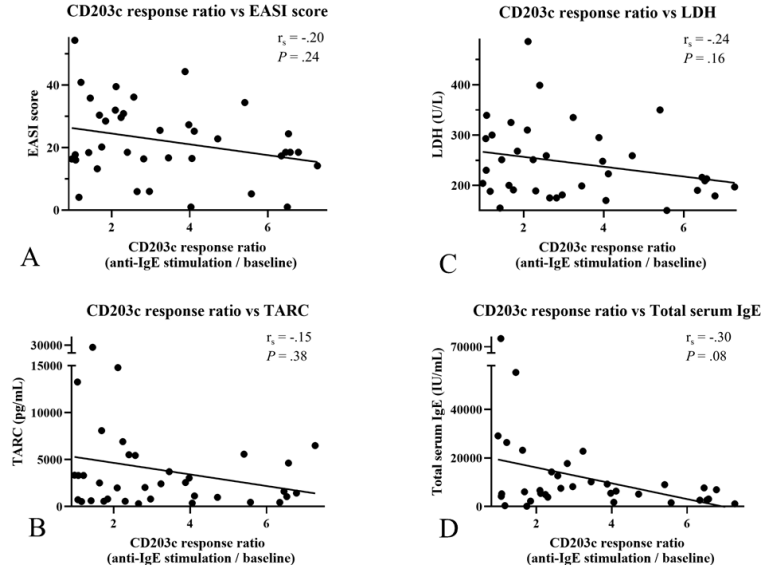

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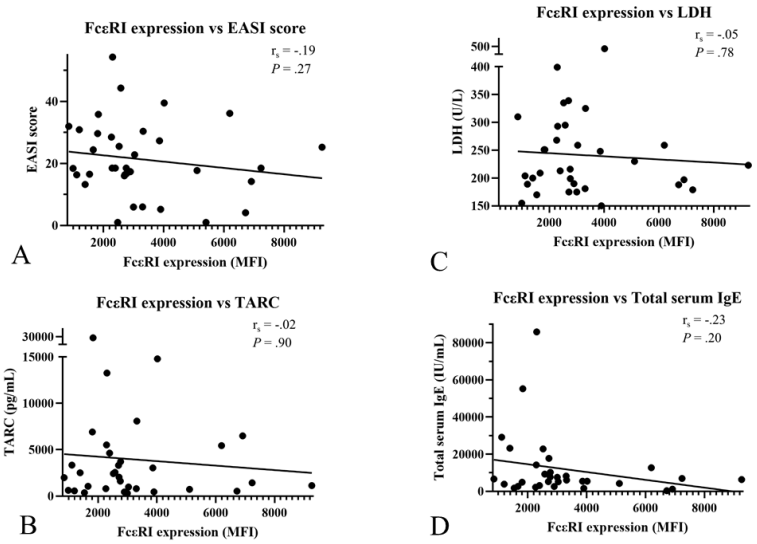


FIGURE S5.

Supplemental figure legends

FIGURE S1. Flow cytometric data analysis. (A) Total leukocyte population. (B) Lymphocyte and monocyte population. (C) Basophil population. FSC, forward scatter; SSC, side scatter; PE, phycoerythrin; PE-Cy7, PE-cyanine dye 7; FITC, fluorescein isothiocyanate.

FIGURE S2. Correlations between baseline CD203c 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.

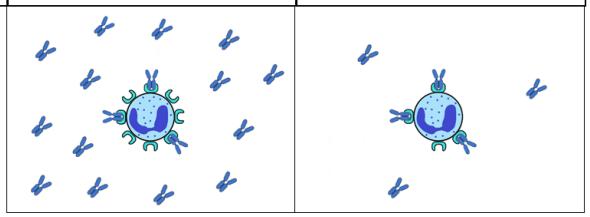
FIGURE S3. Correlations between baseline CD63 and clinical/laboratory factors in AD patients. (A) EASI score. (B) TARC. (C) LDH. (D) Total serum IgE. Statistical analyses were performed using Spearman's rank correlation coefficient.

FIGURE S4. Correlations between CD203c 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.

FIGURE S5. Correlations between FcεRI expression 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.

Graphical abstract

	AD patients	HCs
Total serum IgE	High	Normal
FcεRI expression	High	Normal
Surface-bound IgE	Equivalent	Normal
Baseline CD203c/CD63 expression	↑	→
Response with anti-IgE stimulation	↑ ↑	↑ ↑ ↑ ↑
Response with anti-FcεRI stimulation	↑ ↑ ↑	↑ ↑ ↑



GRAPHICAL ABSTRACT

Highlight

Highlight. Total-serum IgE and FcεRI expression on basophils were higher in AD patients than in HCs, although surface-bound IgE on basophils was equivalent.

Baseline CD203c/CD63 was spontaneously upregulated in AD patients, but responsiveness to anti-IgE stimulation was lower in AD patients than in HCs.

Responsiveness to anti-FcεRI stimulation was higher than that to anti-IgE stimulation in AD patients, contrary to HCs.

-, normal expression; —, mildly increased expression; ——, moderately increased response ratio; ———, severely increased response ratio; ————, extremely increased response ratio.

Supplemental table

Table S1. Clinical characteristics of AD patients and HCs

HCs (n=21)	HCs (n=21)	HCs (n=21)	Normal range
Age, y		29 (26–46)	
Sex	Male	7 (33.3%)	
	Female	14 (66.7%)	
AD patients (n=38)	AD patients (n=38)	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%)	

HCs (n=21)	HCs (n=21)	HCs (n=21)	Normal range
	Allergic conjunctivitis	7 (18%)	
	Urticaria	6 (15.79%)	

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 and complications.

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