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IgE-expressing memory B cells and plasmablasts are increased in blood of children with asthma, food allergy and atopic dermatitis

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Abstract

Despite the critical role of soluble IgE in the pathology of IgE-mediated allergic disease, little is known about abnormalities in the memory B-cells and plasma cells that produce IgE in allergic patients. We here applied a flowcytometric approach to cross-sectionally study blood IgE+ memory B-cells and plasmablasts in 149 children with atopic dermatitis, food allergy and/or asthma, and correlated these to helper-T(h)2 cells and eosinophils. Children with allergic disease had increased numbers of IgE+CD27- and IgE+CD27+ memory B-cells and IgE+ plasmablasts, as well as increased numbers of eosinophils and Th2 cells. IgE+ plasmablast numbers correlated positively with Th2 cell numbers. These findings open new possibilities for diagnosis and monitoring of treatment in patients with allergic diseases.

Key words: Asthma, IgE, memory B-cell, plasmablast, flowcytometry

Introduction

IgE is commonly used in the diagnostic work-up of patients with allergic disease, but little is known about the cells producing it.[1, 2] All antibodies, including IgE, are produced by plasma cells.[3] IgM, IgG and IgA expressing memory B cells and plasma cells can be readily detected in tissue or blood of healthy children and adults. In contrast, IgE-expressing B cells have been enigmatic.[4] Still, based on the presence of IgE transcripts isolated from various tissues and blood of healthy controls and allergic subjects[5-7], the presence of IgE-expressing B cells has been anticipated.[8] We developed a flowcytometric strategy that enabled the detection of IgE-expressing $CD27^{+}CD38^{hi}$ plasmablasts, CD27+ and CD27- memory B cells.[9] Although

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T-cell help is required to generate the former subsets, CD27-IgE+ B cells can be formed independent of cognate T-cell help.[9, 10]

More insights into IgE-expressing B cells and their interactions with other immune cells are needed to better understand the pathogenesis of allergic disease, which could be useful for diagnostic purposes or to uncover targets for treatment. Similar to immunotherapy, anti-IgE antibody treatment can potentially target IgE+ memory B-cells and thereby might resolve the underlying allergy.[11]

We performed a cross-sectional study into IgE-expressing B cells in allergic children and healthy controls. Patients were separated into three groups based on distinct allergic phenotypes of asthma, food allergy and/or atopic dermatitis. We studied IgE+ memory B-cell and IgE+ plasmablast numbers with flow cytometry, their maturation status with molecular studies, and related these to clinical phenotype and other clinical and immunological parameters.

Methods

Peripheral blood samples were obtained from children aged 6-18 years who were recruited from the allergy outpatient clinics (KinderHaven and allergy department) of the Erasmus University Medical Center Rotterdam. All children were recruited from a tertiary referral center for children with allergic disease and were doctor's diagnosed as moderate to severe allergic disease. All children had allergic disease based on clinical examination and confirmed either by atopy skin test (HEP >0.21), specific IgE serum levels (>0.35 IU/L), immune solid-phase allergy chip (ISAC) or, in the case of food-allergy, double blind food provocation test. Based on symptoms, clinical findings and IgE-specificity, children with allergic disease were divided into three groups: 1) children with asthma in absence of food allergy (FA) and atopic dermatitis (AD): 'Asthma', n=68; 2) children with asthma in

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combination with FA and/or AD: 'Asthma + FA/AD', n=48; and 3) children with FA and/or AD in absence of asthma; 'FA/AD', n=33. Age-matched non-allergic controls were recruited among children undergoing surgery in the Sophia Children's Hospital in Rotterdam. Exclusion criteria for the study were: age <6 years, systemic immunosuppressive therapy and immunological diseases, such as immunodeficiencies and auto-immune diseases.

All peripheral blood samples were collected after written informed consent was obtained and with approval of the Medical Ethical Committee of the Erasmus Medical Center, which complies with the Helsinki declaration.

Detailed methods on flow cytometry, molecular analysis of IgE transcripts and statistics can be found in Appendix S1.

Results

Patient characteristics

A total of 149 allergic and 15 non-allergic children were included. All allergic children were sensitized for either aero- or food-allergens, as detailed per group in Table S1.

Increased numbers of eosinophils, T-cells and B-cells in children with allergic disease

Total leukocyte counts were higher in patients with allergic disease than in non-allergic controls (Figure 1A). Similarly, total T- and B-cell numbers were significantly higher in each of the 3 groups with allergic disease than in non-allergic controls (Figure 1B). Total numbers and frequencies of eosinophils were significantly increased in allergic children as compared to non-allergic children (Figure 1C and Figure S2). Leukocyte subsets and total T- and B-cell numbers did not differ between the three allergic groups.

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Absolute CD4+ T-cell numbers and subsets within CD4+ T-cells were significantly higher in children with allergic disease than in non-allergic children (Figure 1D). Still, frequencies of CD4 T-cells within CD3+ T-cell were not different from healthy children (Figure 1E). In addition, frequencies of Th1 and Th2 subsets within CD4+ T cells were not different between healthy children and children with allergic disease (Figure 1F). As a result, we did not observe a difference in Th2/Th1 cell ratios (Figure 1G) between patients and controls.

Increased numbers of circulating IgE+ memory B-cells and IgE producing plasmablasts

IgE+ memory B-cells were defined within the $CD19^{+}CD38^{dim}$ fraction and IgE+ plasmablasts within the $CD19+CD38+CD27^{hi}$ fraction following exclusion of CD19+ B-cells expressing IgM, IgD, IgA or IgG (Figure 2A).[9]

Median cell numbers of both CD27+IgE+ and CD27-IgE+ memory B-cells were significantly higher in all three groups of allergic children than in non-allergic controls (Figure 2B). In addition, IgE+ plasmablast numbers were significantly increased in allergic children (Figure 2C). IgE+ memory B-cell and IgE+ plasmablast numbers did not significantly differ between patient groups. The increases in IgE+ B-cell numbers in allergic children were not only the result of higher total B-cell counts, because their relative numbers within CD19+ B cells were also significantly higher in all patient groups (Figure 2D,E).

To investigate whether the increased numbers constituted normal plasmablasts, we studied the levels of somatic hypermutation (SHM) in IgE transcripts of sorted cells from allergic children (n=43 unique transcripts) and from non-allergic controls (n=34 unique transcripts). Transcripts from patients carried fewer mutations in their *IGHV* genes than controls (Figure 2F). As a measure for antigen-driven selection of the mutations in the *IGHV* genes, we analyzed the selection for replacement mutations in the complementarity

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determining regions (CDR) with the BASELINe program. Transcripts from healthy controls ($P_{CDR/FWR}$ -0.48) nor from children with allergic disease ($P_{CDR/FWR}$ 0.06) showed more replacement mutations than expected by random chance in the CDR regions, i.e. absence of positive selection (Figure 2G). Thus, the higher numbers of circulating IgE-expressing B-cells in children with allergic disease did not show molecular signs of enhanced antigen receptor maturation.

Since type 2 cytokines produced by helper-T(h) cells are important for IgE class switch recombination (CSR) and children with allergic disease had increased numbers of IgE+ B-cells, we investigated the correlation between Th cells and IgE+ B-cells. We observed a correlation between IgE+ plasmablast numbers and Th2 cells (r 0.271; P <0.01), whereas there was no correlation between IgE+ memory B-cell subsets and Th2 cells (Figure 2H).

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Discussion

In the present study we extended the immunological phenotype of allergic children. In addition to Th2 cells, eosinophils, basophils and serum IgE, we found increased numbers of circulating IgE+ memory cells and IgE+ plasmablasts, and a positive correlation between IgE+ plasmablasts and Th2 cell numbers.

In our patient group, type 2 sensitization was confirmed by high IgE serum levels, sensitization to various aero- and food allergens, high numbers of eosinophils in blood and increased Th2 cell numbers.

Our findings of increased numbers of circulating IgE+ memory B cells extend previous observations.[9, 12, 13] Importantly, our flowcytometric gating strategy enabled exclusion of IgE-binding cells from the true IgE-expressing B cells, which we confirmed by the presence of mature IgE transcripts. We showed that this occurs in children, and is independent of the allergic phenotype. A cause for the increased numbers of circulating IgE+ memory B cells could be the chronic inflammation. This is specifically thought to occur in patients with atopic dermatitis, and to some extent also in asthma. Here we found increased numbers of both CD27+ and CD27- IgE-expressing memory B cells in allergic patients. It has been shown that CD27- IgE+ memory B-cells display a limited proliferation history and lower SHM levels in their immunoglobulin genes[9], which suggest that they originate from mucosal tissue, analogous to CD27-IgA+ memory B-cells.[14] This is supported by previous detections of IgE transcripts and Sμ-Sε switch circles in nasal tissue of patients with allergic rhinitis and in bronchial tissue of patients with allergic asthma.[8, 15] Several studies have shown that sensitized individuals who are exposed to allergens via nasal tissue or the respiratory tract, display a substantial increase in systemic serum IgE levels, thus indicating the direct activation of an established local pool of IgE memory cells with defined specificities as an underlying mechanism.[16]

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We did not observe a correlation between IgE+ memory B-cell and Th2 cell numbers, but we did find a positive correlation between Th2 cells and IgE+ plasmablasts. Previous studies show that type 2 innate lymphoid cells (ILC2) might play an important role in the local CSR towards IgE.[17, 18] Furthermore expansions of ILC2 populations have been observed in allergic disease and are known to produce type 2 cytokines and ILC2 cells facilitate sensitization to local, but not systemic Th2 inducing allergen exposures.[19] Next to that, T follicular helper (Tfh) cells have been linked to IgE class switching through the production of IL-4.[20, 21] Tfh cells express the B-cell follicle homing receptor CXCR5 and mainly reside in germinal centers. Thus possibly ILC2 cells might be important in local IgE CSR, Tfh cells in germinal center IgE CSR, whereas Th2 cells are important in plasma cell differentiation. In future studies it would be interesting to study ILC2 cells in mucosal tissue and correlate these to IgE+ memory B-cell numbers.

It is uncertain if circulating IgE+ memory B-cells sustain the underlying allergy, but in vitro stimulated IgE+ memory B-cells can differentiate into IgE+ plasma cells.[9] Therefore it is possible that increased circulating IgE+ memory B-cells actively contribute to allergic disease.[22] To achieve desensitization and possibly even cure allergic disease, it might therefore be important to develop therapies to reduce IgE+ memory B-cells. Omalizumab treatment in humans has shown to decrease serum IgE in patients with IgE-mediated asthma[23], however it mainly targets soluble IgE. This is not the case for Quilizimab, which only recognizes surface-expressed IgE on B cells[11], and has shown to deplete IgE producing B-cells in mouse studies.[24] Although human clinical trials with Quilizimab reduced IgE serum levels for 6 months after treatment cessation, the effects on clinical parameters were variable.[25, 26] Our present findings can possibly contribute to patient selection and treatment monitoring of anti-IgE treatment.

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Author contributions

JJH, JCdJ, JJMvD and MCvZ designed research. JJH and LR performed research. JJH, LR and MCvZ analyzed data. NJA, SGP, JCdJ and GJD evaluated and included patients in the study. JJH and MCvZ wrote the paper, and all authors commented on the paper and approved the final version.

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Figure legends (n=2)

Figure 1. Absolute numbers and frequencies of leukocyte subsets and CD4+ helper-T(h) subsets. **A.** White blood cell count. **B.** Absolute numbers of T cells and B cells. **C.** Percentages of granulocyte subsets and monocytes within total CD45+ cells. **D.** Absolute cell numbers of CD4+ T-cells, Th1 and Th2 cells **E.** Percentage of CD4+ T-cells within total CD3+ T-cells. **F.** Percentage of Th1 and Th2 cells within total CD3+ T-cells. **G.** ratio of Th2/Th1 cells. Each dot represents one individual and red lines indicate median values. Grey surface represent reference values. Statistical analysis was performed with Mann Whitney *U* test. *P<0.05, **P<0.01, ***P<0.001

Figure 2. IgE+ B-cell subsets. **A.** Representative flowcytometry image and gating strategy of IgE+ memory B-cells. **B.** Absolute numbers and percentages of IgE+ memory B-cells. **C.** Absolute numbers and percentages of IgE+ plasmablasts. **D.** Somatic hypermutation (SHM) levels in the *IGHV* gene of IgE+ plasmablasts from healthy children and children with allergic disease (n=5 children each). **E.** Selection for replacement mutations in the CDR (red) and FR (blue) regions. **F.** Correlation of Th2 cell numbers with IgE+CD27-, IgE+CD27+ and IgE+ plasmablasts in children with allergic disease. In panels B, C and F each dot represents one individual; red lines indicate median values; and black lines in panel F indicate linear correlations. Statistical analysis was performed with Mann Whitney *U* test. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001. Correlation was calculated with Spearman R.

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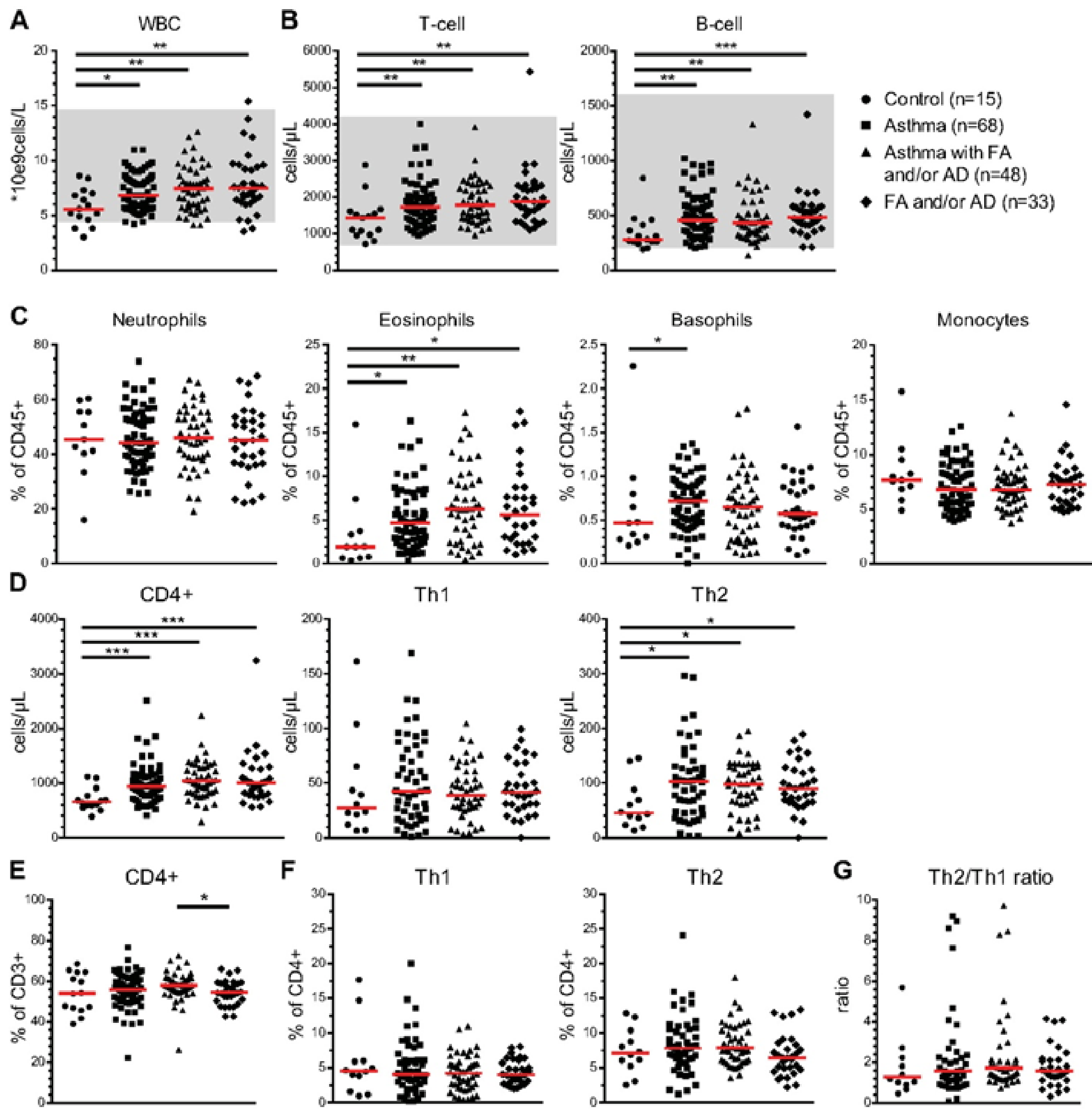

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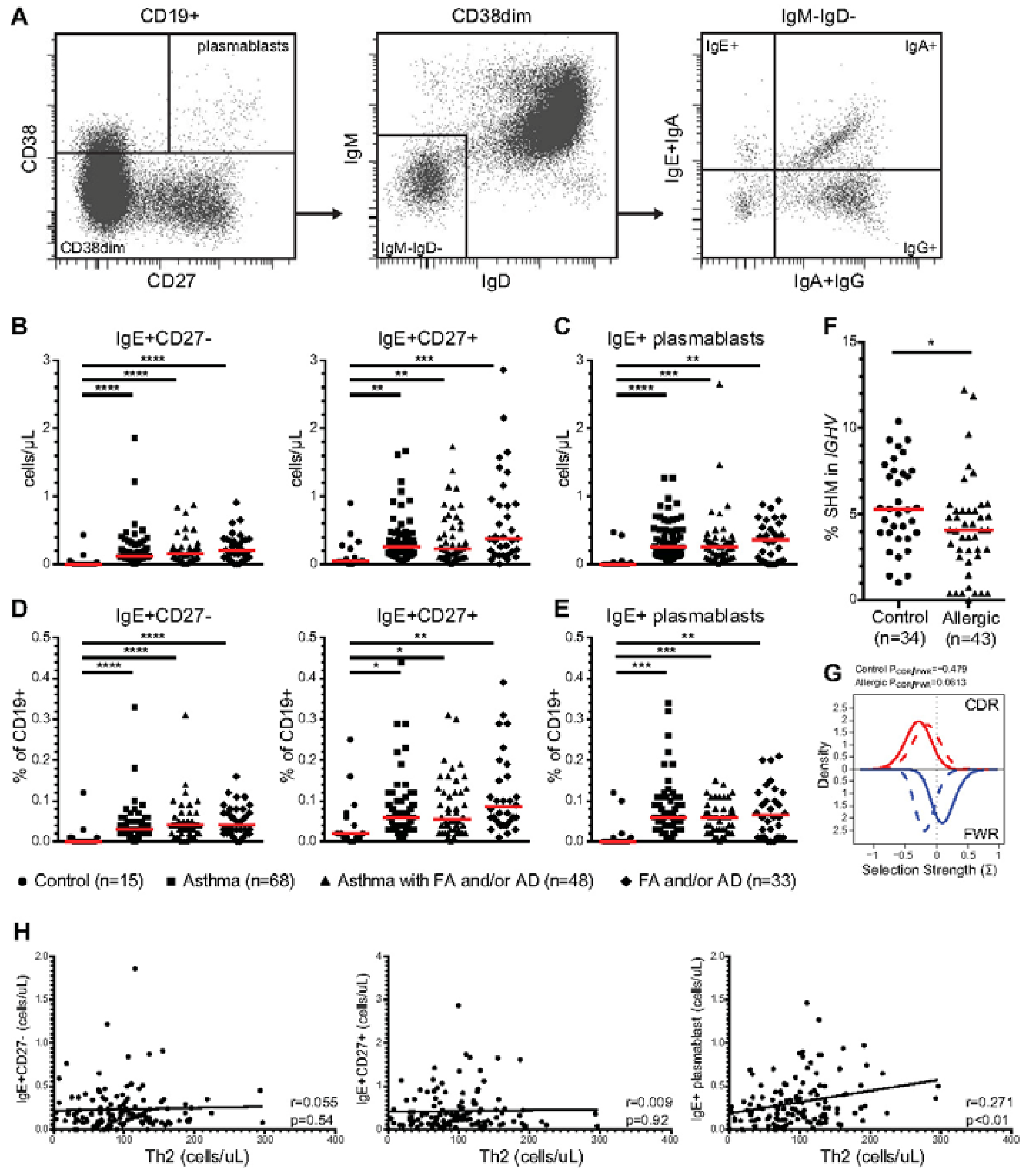

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