

Basophil activation by anti-double-stranded DNA IgE antibody enhances B cell differentiation in systemic lupus erythematosus

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October 27, 2022

Abstract

Objects Recently, the involvement of basophils and IgE-type autoantibodies in the pathogenesis of systemic lupus erythematosus (SLE) has been elucidated using mouse models; however, few studies have been conducted in humans. In this study, the role of basophils and anti-double-stranded DNA (dsDNA) IgE in SLE was examined using human samples. **Methods** The correlation between disease activity and serum levels of anti-dsDNA IgE in SLE was evaluated using enzyme-linked immunosorbent assay. Cytokines produced by IgE-stimulated basophils from healthy subjects were assessed using RNA sequences. The interaction of basophils and B cells to promote B cell differentiation was investigated using a co-culture system. The ability of basophils from patients with SLE with anti-dsDNA IgE to create cytokines promoting B cell differentiation in response to dsDNA was examined using real-time polymerase chain reaction. **Results** Anti-dsDNA IgE levels in the serum of patients with SLE correlated with disease activity. Healthy donor basophils produced IL-3, IL-4, and TGF- β 1 after anti-IgE stimulation. Co-culture of B cells with anti-IgE-stimulated basophils increased plasmablasts which were cancelled by neutralizing IL-4. After encountering the antigen, basophils released IL-4 more quickly than follicular helper T cells. Basophils isolated from patients with anti-dsDNA IgE promoted IL-4 expression by adding dsDNA. **Conclusions** These results suggest that basophils contribute to the pathogenesis of SLE by promoting B cell differentiation via dsDNA-specific IgE in patients similar to the process described in mouse models.

Title

Basophil activation by anti-double-stranded DNA IgE antibody enhances B cell differentiation in systemic lupus erythematosus

Keywords

Basophil, IgE, Autoimmunity, B cell

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Competing interests

None declared.

Grant

Japan Society for the Promotion of Science, Grant-in-Aid for Scientific Research (C)/19K08910.

Word count

3203 words

Abstract

Objects

Recently, the involvement of basophils and IgE-type autoantibodies in the pathogenesis of systemic lupus erythematosus (SLE) has been elucidated using mouse models; however, few studies have been conducted in humans. In this study, the role of basophils and anti-double-stranded DNA (dsDNA) IgE in SLE was examined using human samples.

Methods

The correlation between disease activity and serum levels of anti-dsDNA IgE in SLE was evaluated using enzyme-linked immunosorbent assay. Cytokines produced by IgE-stimulated basophils from healthy subjects were assessed using RNA sequences. The interaction of basophils and B cells to promote B cell differentiation was investigated using a co-culture system. The ability of basophils from patients with SLE with anti-dsDNA IgE to create cytokines promoting B cell differentiation in response to dsDNA was examined using real-time polymerase chain reaction.

Results

Anti-dsDNA IgE levels in the serum of patients with SLE correlated with disease activity. Healthy donor basophils produced IL-3, IL-4, and TGF- β 1 after anti-IgE stimulation. Co-culture of B cells with anti-IgE-stimulated basophils increased plasmablasts which were cancelled by neutralizing IL-4. After encountering the antigen, basophils released IL-4 more quickly than follicular helper T cells. Basophils isolated from patients with anti-dsDNA IgE promoted IL-4 expression by adding dsDNA.

Conclusions

These results suggest that basophils contribute to the pathogenesis of SLE by promoting B cell differentiation via dsDNA-specific IgE in patients similar to the process described in mouse models.

Introduction

Systemic lupus erythematosus (SLE) is a complicated autoimmune disease with symptoms in multiple organs and a varied clinical course(1). Life expectancy, the primary goal in SLE, was significantly improved with the introduction of glucocorticoids in the 1960s. Organ prognosis, the secondary treatment goal, was dramatically improved with the advent of immunosuppressive agents targeting acquired immunity commencing with cyclophosphamide in the 1990s. In recent years, the efficacy of molecularly targeted therapy to specific cytokines, including anifrolumab, a monoclonal antibody to type I interferon receptor subunit 1, and belimumab, a B cell activating factor (BAFF) monoclonal antibody, has been reported(2–4). However, there remain some patients in whom the progress of organ damage cannot be controlled even with current treatment(5), suggesting that certain cell populations or cytokine signaling may exist that are not controlled

by currently available treatments. Given the heterogeneity of SLE, a multimodal approach to therapy is required.

Basophils, classified as myeloid cells, have rarely been discussed for their role outside of allergic disease and parasitic infection in part due to their small absolute numbers. However, recently, their diverse immune functions have been revealed. In 2008 and 2009, several novel immune functions of mouse basophils were reported, including memory enhancement of humoral immunity (6), induction of Th2 differentiation (7,8), and antigen presentation via major histocompatibility complex class II molecules (7,9). Given this mouse model background, basophils' involvement in the pathogenesis of human inflammatory and autoimmune diseases has begun to be explored (10).

Simultaneously, it was reported that autoantibodies of the IgE class, the main stimulator of basophils, are present in the sera of patients with various autoimmune diseases, and their titers correlate with disease activity (11). In 2006, anti-nuclear IgE antibodies were reported in patients with SLE (12). This was followed by a report in 2010 of a correlation between IgE-type autoantibody titers against double-stranded DNA (dsDNA) in patients' serum and disease activity in 2010 (13). Since then, the possible involvement of IgE-type autoantibodies/basophils in lupus nephritis has been proposed using mouse models of SLE (13,14). The expected mechanism is activation of basophils by IgE-type immune complexes, which interact with B cells and increase plasma cells in secondary lymphoid tissue thereby promoting autoantibody production (13). These findings in the mouse model suggest that IgE-type autoantibodies/basophils may constitute a novel immune response axis in SLE. However, the pathogenetic mechanism of basophils and IgE-type autoantibodies in humans remains to be elucidated.

This study attempted to clarify whether the pathophysiological model proposed in mice can be applied to human SLE. This study examined whether healthy human basophils activated by IgE can promote B cell differentiation, and whether basophils from patients with SLE were similarly activated in response to dsDNA.

Materials and Methods

Patients

A total of 37 Japanese patients with SLE at the Kyushu University Hospital were studied. The patients fulfilled the classification criteria for the systemic lupus international collaborating clinics classification criteria 2012. All patient samples were collected following written informed consent according to local ethics policy guidelines and the Declaration of Helsinki. Information obtained from the patients' medical records included demographic data, clinical manifestations, laboratory findings, and medications.

Human basophil and B cell isolation

Human blood samples were obtained from healthy donors in heparin tubes (Terumo Corporation). Peripheral blood mononuclear cells (PBMCs) were separated by a density gradient method using Lymphoprep (#1114545, Axis-Shield). Basophils were isolated by negative selection from PBMCs using the Human Basophils Enrichment kit (#17969, Stemcell Technologies). B cells were isolated from PBMCs by negative selection using the B Cell Isolation Kit II (#130-091-151, Miltenyi Biotec). Both basophils and B cells were greater than 95% pure.

Fluorescence-activated cell sorting analysis

PBMCs or cultured cells were washed with phosphate-buffered saline (PBS) and resuspended with 2% fetal bovine serum (FBS) with 2 mM EDTA (Thermo Fisher Scientific) in PBS. The cells were incubated with fluorescence-labeled antibodies for 30 minutes at 4 C. After washing, dead cells were stained using propidium iodide (#421301 BioLegend). Live-cell analysis was performed for each cell subset using Attune Flow Cytometer (Thermo Fisher Scientific) according to the following gates: basophils (FcεRI⁺CD123⁺ cells in lymphocyte gate), plasmablasts (CD19⁺CD27^{high}CD38^{high}CD20^{low} cells in lymphocyte gate), and follicular helper T cells (Tfh) (CD4⁺CD45RA⁻CXCR5⁺PD-1^{high} cells in lymphocyte gate). Data were analyzed using

FlowJo v10.5 software (BD Biosciences). Fluorescent labeled antibodies used in the assay are summarized in Supplemental Table 1.

Immunohistochemistry

To detect basophils in a lymph node, formalin-fixed, paraffin-embedded lymph node sections of the patients were deparaffinized and rehydrated using xylene and ethanol. Heat-induced epitope retrieval was then performed by microwaving samples in a Tris-based antigen unmasking solution (pH 9.0) (#H-3301, Vector Laboratories, Inc). Endogenous peroxidase activity was blocked using 3% H2O2 for 5 minutes. Nonspecific staining was blocked by incubation in Protein Block Serum-Free (#X0909, Dako) for 10 minutes at room temperature. The slides were stained for 18 hours at 4 with a mouse anti-basophil (2D7) antibody (1:50) (#ab155577, Abcam) and then incubated in EnVision+ Dual Link System-HRP (#K4063, Dako). The sections were stained with the ImmPACT DAB Substrate kit, Peroxidase (#SK-4105, VECTOR). Finally, slides were counterstained with hematoxylin.

Real-time quantitative polymerase chain reaction

Total RNA was extracted using Trizol-LS Reagent (#10296010, Invitrogen) following the manufacturer's recommendations. Synthesis of cDNA was performed using the iScript reverse transcriptase kit (#1708890, Bio-Rad), and real-time polymerase chain reaction (PCR) was performed using the iTaq Universal SYBR Green Supermix kit (#1725121, Bio-Rad) on StepOnePlus (Applied Biosystems). The primers used for real-time PCR are noted in Supplemental Table 2. Basophil reactions were normalized to β -actin level, and B cell reactions to β 2-microglobulin to evaluate relative gene expression.

RNA sequence

Basophils isolated from six healthy donors were cultured for three hours (50,000 basophils per 200 μ L medium) with or without anti-human IgE antibody (500 ng/ml) and then harvested. For purity, harvested cells were stained and sorted directly into Trizol-LS (#10296010, Invitrogen) by BD FACSAria III (BD Biosciences). Total RNA was extracted from the lysate using a Direct-zol RNA MicroPrep (#R2060, ZYMO RESEARCH), and a quality check was performed using 4200 TapeStation (Agilent). Library preparation, sequencing, and analysis (including calculations of differentially expressed genes) were performed at the Transcriptomics, Medical Institute of Bioregulation, Kyushu University. Differentially expressed genes, with a false discovery rate < 0.1, were extracted for heatmaps, and the KEGG_CYTOKINE_CYTOKINE_RECEPTOR_INTERACTION gene set was used to select cytokine, chemokine, and each receptor.

Co-culture assay of Basophils and B cells

RPMI 1640 supplemented with 10% FBS (#172012-500ML, Sigma-Aldrich) and Penicillin-Streptomycin Solution (#168-23191, FUJIFILM Wako Pure Chemical) were used for the primary cell culture. Basophils and B cells were co-cultured at a ratio of 1:2 (50,000 basophils with 100,000 B cells) in 96-well flat-bottom plates with 200 μ L medium containing human IL-2 (100 U/ml; #202-IL-050, R&D), human CD40L (100 ng/ml; #591706, BioLegend), and human IL-21 (50 ng/ml; #571204, BioLegend) per well. 500 ng/ml of anti-human IgE antibody (#ME-80A-24A, ICL) was added at the start of incubation. After seven days, cultured cells were harvested and analyzed by fluorescence-activated cell sorting (FACS).

In the cytokine neutralization assay, neutralizing antibodies, including anti-human IL-3 antibody (#MAB603100, R&D), anti-human IL-4 antibody (#500701, Biolegend), and anti-transforming growth factor- β 1 (TGF- β 1) antibody (#MA1-21595, Invitrogen) were added at the initiation of the co-culture system.

Anti-double-stranded DNA (dsDNA) IgE enzyme-linked immunosorbent assay (ELISA)

dsDNA coated plates (#DD037G, Calbiotech) were used to measure human anti-dsDNA IgE. Serum was diluted 1:10 with PBS containing 10% FBS and incubated for 2 hours at room temperature. The plates were then washed, incubated for 2 hours at room temperature with HRP-conjugated anti-IgE antibodies

(#ME-80P-24A, ICL), washed again, developed using TMB substrate (#555214, BD Biosciences), and the optical density at 450 nm was measured.

Basophil cytokine production assay

In order to measure anti-IgE-stimulated basophil-derived cytokine, basophils were cultured for seven days (50,000 basophils per well) in 96-well flat-bottom plates with 200 μ L medium with or without anti-human IgE antibody (500 ng/ml). The supernatant was then measured with DuoSet ELISA kit for each cytokine including IL-3, IL-4, IL-6, IL-13, BAFF, and TGF β 1 (#DY203-05, #DY204-05, #DY206-05, #DY213-05, #DY2106-05, #DY240-05, R&D).

Basophils were incubated for 3 hours with or without dsDNA (0.5 μ g/ml) in order to measure IL-4 mRNA, and then real-time PCR was performed on the cells.

Statistical analysis

The specific statistical tests used are noted in the figure legends. Statistical analyses were conducted using the Python statistics library (version 3.4) for Spearman's rank correlation coefficient, Student t-test, analysis of variance, and Kruskal–Wallis test. The Steel test was implemented using R (version 4.1.2). P values < 0.05 were considered statistically significant.

Results

Disease activity in SLE correlates with anti-dsDNA IgE titer

Since there are reports on the correlation between anti-dsDNA IgE titer and disease activity (SLEDAI) in serum of patients with SLE(13,15,16), the first step was to confirm their reproducibility. In this study of 37 patients, anti-dsDNA IgE levels correlated with anti-dsDNA IgG titers ($r = 0.608$; $p = 5.1 \times 10^{-5}$) and tended to be inversely correlated with C3 ($r = -2.8$, $P = 0.09$) (Figures 1A and 1B). Additionally, anti-dsDNA IgE levels correlated with SLEDAI ($r = 0.554$; $p = 3.1 \times 10^{-4}$) (Figure 1C). These findings suggest that anti-dsDNA IgE is positively associated with disease activity in SLE.

Human basophilic produce IL-4, TGF- β 1, and IL-3 β IgE cross-linking

To examine the involvement of anti-dsDNA IgE in the pathogenesis of SLE, the basophil, which has a high-affinity IgE receptor, Fc ϵ RI, was studied. Nicolas Charles et al. proposed a mouse model in which basophils stimulated by dsDNA-specific IgE produce IL-4, IL-6, and BAFF to differentiate B cells into plasma cells. These plasma cells then produce IgE which restimulates basophils(17). This study examined whether IgE-stimulated basophils affect B cell differentiation in humans by investigating which cytokines are produced by human basophils following IgE cross-linking.

The Fc portion of Fc ϵ RI and IgE bind strongly to each other with a low dissociation constant(18); therefore, IgE remains bound to the surface of basophils after isolation from human peripheral blood(19). Using this property, antigen-IgE-Fc ϵ RI cross-linking was mimicked by adding anti-IgE to basophils isolated from human peripheral blood and measuring which cytokines the basophils produced. Based on reports that anti-IgE stimulation of mouse or human basophils produce IL-4, IL-6, and BAFF(13,20–22), this study examined whether similar behavior could be observed in humans. The ELISA results found IL-4 to be expressed but not IL-6 or BAFF (Figure 2A).

There are few studies regarding human basophils stimulated with anti-IgE. To investigate whether basophils produce unreported factors that affect B cell differentiation, RNA sequencing of anti-IgE-stimulated healthy basophils ($n = 6$) was performed. These results found that some of the basophil's cytokine-related genes were changed by anti-IgE stimulation (Figure 2B). Based on previous reports (Supplemental Table 3), IL3, IL13, TGF β 1, and taxillin α (TXLNA) were extracted as cytokine genes that may affect B cell differentiation and proliferation other than IL-4, IL-6, and BAFF (Figure 2C).

Next, the protein expression of these genes using ELISA was studied and it was determined that TGF- β 1, but not IL-13, was released (Figure 2D). Since it has been reported that IL-3 binds to its receptor quickly

after being released from basophils, making it difficult to measure by ELISA (23), the expression of IL-3 by real-time PCR was performed, and it was determined that IL-3 was expressed (Figure 2D). TXLNA is a gene encoding IL-14, and since there is no commercially available ELISA kit, its expression was studied by real-time PCR. However, no increase in expression was observed upon anti-IgE stimulation (Figure 2D). Given these results, IL-4, TGF- β 1, and IL-3 derived from anti-IgE-stimulated basophils were considered as candidate mediators for promoting B cell differentiation and proliferation.

B cell differentiation into plasmablast assisted by co-culture with anti-IgE-stimulated basophils.

To determine the effect of human basophils on B cell differentiation, the differentiation of B cells into plasmablasts was examined in a co-culture assay. B cells are known to differentiate into plasmablasts when in contact with the Tfh environment (24). Co-culture of B cells and anti-IgE-stimulated basophils in the Tfh environment revealed an increasing absolute number of plasmablasts (CD19⁺CD27^{high}CD38^{high}) and an increased ratio to CD19⁺B cells (Figures 3A and 3B). CD19⁺CD27^{high}CD38^{high} cells demonstrated increased PR domain containing 1 expression and decreased paired box 5 expression on real-time PCR in addition to decreased CD20 expression on FACS analysis (Figure 3C) consistent with plasmablast differentiation from naïve B cell (25). These results indicate that co-culture with anti-IgE-stimulated basophils enhances B cell development into plasmablasts in the Tfh environment.

Basophil-derived IL-4 supports plasmablast differentiation.

Cytokine neutralization experiments were performed to determine which basophil-derived factor contributed to promoting plasmablast differentiation. By adding the respective neutralizing antibodies to the B cell and IgE-stimulated basophil co-culture system, it was determined that neutralizing IL-3 and TGF- β 1 did not change plasmablast differentiation. However, neutralizing IL-4 suppressed differentiation into plasmablasts significantly (Figures 4A and B). This indicates that IL-4 released by basophils supports plasmablast differentiation of B cells.

Next, cell separation experiments were performed using transwells to evaluate whether a liquid factor, including IL-4, is the only factor involved in plasmablast differentiation. Direct co-culture of basophils and B cells, as in the prior experiment, increased the number of plasmablasts compared to B cells alone. However, the number of plasmablasts was decreased when B cells were co-cultured in a transwell with basophils compared to direct co-culture (Supplemental Figure 1). This suggests that the liquid factor is not the only supporter of plasmablast differentiation but that there are factors that work by cell-to-cell contact. The RNA sequence was reanalyzed to search for candidate surface molecules on basophils that enhance B cell differentiation, but none were found. It therefore appears that other factors besides liquid factors may affect basophil-induced B cells, but basophil-derived IL-4 promotes plasmablast differentiation.

Basophils release IL-4 more rapidly than Tfh upon encountering the antigen

The IL-4-producing capacity of basophil was compared to that of the Tfh. IL-4 released from basophils stimulated with anti-IgE and Tfh with CD3/28 beads (mimicking antigen-presenting cell stimulation) was evaluated by ELISA. It was found that basophils primarily produced IL-4 up to 16 hours post-stimulation, after which Tfh production of IL-4 predominated (Figure 4C). Thus, basophils would produce IL-4 more quickly than Tfh.

Basophils can migrate to lymph nodes in patients with SLE

Although basophils and mast cells have similar functions, basophils have one characteristic that mast cells lack. They circulate peripherally throughout the body and settle in lymph nodes. Pellefigues and Charles et al. reported that the basophils of patients with SLE settle into lymph nodes via CXCR4 and CD62L (13,14). The expression of CXCR4 and CD62L on basophils from healthy subjects was reconfirmed by FACS (Figure 5A), and the presence of basophils in the lymph node of a patient with SLE was confirmed using immunohistochemistry with an anti-2D7 antibody which binds to the cytoplasmic granules of basophils (Figure 5B) (26). These findings suggest that basophils may be present in the lymph nodes of patients with SLE and interact with B cells.

Basophils from patients with SLE having anti-dsDNA IgE antibodies produce IL-4 in response to dsDNA

Next, we examined whether basophils derived from patients with SLE produce IL-4 via dsDNA-specific IgE. Since IgE is routinely bound to the surface of basophils, it was hypothesized that dsDNA-specific IgE was also attached to basophils isolated from patients with high anti-dsDNA IgE levels. To test the reaction of patients' basophils to dsDNA, dsDNA was added to basophils from patients with SLE and IL-4 expression using real-time PCR was measured. Anti-dsDNA IgE-positive patients were defined as those having an OD 450 value > 0.2 on ELISA. This experiment noted increased IL-4 production in basophils from anti-dsDNA IgE-positive patients compared to anti-dsDNA IgE-negative patients (Figure 6A). To exclude the slight possibility that basophils are stimulated via small amounts of IgG remaining on their surface(27), patients who only had anti-dsDNA IgG were compared to those who had both anti-dsDNA IgG and IgE. It was determined that basophils from patients with only anti-dsDNA IgG expressed less IL-4 than those with both anti-dsDNA IgG/IgE (Figure 6B), suggesting that basophils do not promote IL-4 production through anti-dsDNA IgG. In summary, basophils in patients with SLE react to dsDNA and produce IL-4 via dsDNA-specific IgE.

Discussion

This study found that human basophils produce IL-4 through IgE stimulation and promote differentiation of B cells into plasmablasts in the Tfh environment. Additionally, it was found that basophils from patients with SLE with dsDNA-specific IgE expressed IL-4 in response to dsDNA. These findings suggest that basophils can play an important role in the pathogenesis of SLE by promoting B cell differentiation via dsDNA-specific IgE in humans, similar to what has been described in mouse models.

This study noted that IL-4 released by IgE-stimulated basophils promoted plasmablast differentiation when healthy basophils were co-cultured with B cells. The co-culture system utilized IL-2, IL-21, and CD40L which were associated with Tfh. Tfh is the essential cell for B cells to differentiate into plasmablasts, and, like basophils, produces IL-4(28). In this study, when the IL-4 producing capacity of basophils and Tfh were compared, the basophil produced more IL-4 than the Tfh early on following antigen stimulation. It has been reported that activated B cells are the first to be affected by IL-4 stimulation during plasmablast differentiation. Subsequently some activated B cells lose their IL-4 response and are destined to become plasmablasts(28). This process suggests that IL-4 is important relatively early in the plasmablast differentiation process. Therefore, basophil-produced IL-4 may impact B cells in the early phases of plasmablast development when also in the presence of Tfh.

This study demonstrated that basophils react differently to dsDNA in patients with SLE depending on the presence or absence of dsDNA-specific IgE. This finding implies that the pathogenesis of SLE can be in part dependent on IgE-dsDNA and can be stratified according to its presence or absence. Omalizumab, a recombinant humanized monoclonal antibody against IgE, has shown efficacy in allergic asthma and chronic urticaria by neutralizing serum IgE(29,30). As for SLE, a proof of concept study investigated the efficacy and safety of omalizumab in patients with specific IgE against anti-dsDNA, anti-sm, and anti-SS-A and found improvement in SLEDAI-2K after 16 weeks of treatment(31). Because it has few side effects and is expected to be an effective therapeutic agent, especially for SLE that is polarizing to type 2 immunity, further investigation is needed.

One of the limitations of this study was that it was not possible to research the function of plasmablasts induced by co-culture with basophils. In particular, it was not possible to determine its ability to produce IgE. It is known that IL-4 acts on B cells and promotes activation-induced cytidine deaminase transcription resulting in a class switch to IgE(32,33). One hypothesis that was considered in this study was that basophil IL-4 synthesis might stimulate B cells to produce more autoreactive IgE which would worsen SLE. However, it was not possible to prove this hypothesis, because IgE adhering to the basophil surface prevented the use of an ELISA assay for IgE from B cells, and anti-IgE used for IgE stimulation hindered the FACS and ELISA systems. A future study will examine IgE production capacity using other measurement systems.

In summary, this study found that cross-linking of dsDNA to basophils with dsDNA-specific IgE on their surface stimulated IL-4 production which stimulated plasmablast differentiation of B cells. These results suggest that basophils may play a role in the pathogenesis of SLE and may lead to the creation of new therapeutic targets.

Acknowledgement

The authors appreciate the patients and healthy volunteers who participated in this research. We thank Professor Kaori Tanaka in Transcriptomics, Medical Institute of Bioregulation, Kyushu University, for assistance with RNA sequence experiments. We thank Enago (www.enago.com) for the English language editing. This study was supported by Japan Society for the Promotion of Science, Grant-in-Aid for Scientific Research (C)/19K08910.

Figure legend

Figure 1

Anti-dsDNA IgE level correlates with SLE disease activity. Correlation of serum anti-dsDNA IgE levels in patients with SLE (n=37) between anti-dsDNA IgG (A), C3 (B) and SLEDAI (C). Anti-dsDNA IgE was measured by ELISA. Statistical significances are determined by Spearman test. dsDNA, double-stranded DNA; SLE, systemic lupus erythematosus; SLEDAI, SLE disease activity index.

Figure 2

Anti-IgE stimulation increases human basophil production of IL-4, TGF- β 1, and IL-3. (A) IL-4, IL-6, and BAFF levels from basophils stimulated with or without anti-IgE. (B) Heatmap for basophil cytokines and receptors changed by anti-IgE stimulation in healthy subjects (n = 6). (C) Volcano plot showing basophil genes altered by anti-IgE stimulation that affect B cell development. (D) The protein of TGF- β 1 and IL-13 and the mRNA of IL3 and TXLNA expression levels of basophil with anti-IgE stimulation. Data are shown as the mean \pm SEM, and statistical significances are determined by Student's t-test. TGF- β 1, transforming growth factor- β 1; TXLNA, taxilin alpha; SEM, standard error of the mean; ND, not detected.

Figure 3

Plasmablast differentiation of B cells is promoted by co-culture with anti-IgE-stimulated basophil. (A and B) Plasmablasts (CD19⁺CD27^{high}CD38^{high}) in co-culture assay of basophils and B cells. One representative FACS plot (A), cumulative absolute numbers and rates in CD19⁺ B cells (B) are shown. (C) Surface marker (CD20) and master genes (PRDM1 and PAX5) expression in plasmablasts and naive B cells. Data are shown as the mean \pm SEM. Statistical significances are determined by one-way ANOVA followed by Turkey post hoc test for multi-group comparisons, ***p<0.0005, or Student's t-test. PRDM1, PR domain containing 1; PAX5, paired Bbox 5; SEM, standard error of the mean; ANOVA, analysis of variance.

Figure 4

IL-4 derived from anti-IgE-stimulated basophils augments B cell differentiation into plasmablast. (A and B) Plasmablasts in the cytokines-neutralizing assay of B cells and anti-IgE stimulated. One representative FACS plot (A), cumulative absolute numbers and rates in CD19⁺ B cells (B) are shown. Data are shown as the mean \pm SEM. Statistical significances are determined by one-way ANOVA followed by Turkey post hoc test, ***p<0.0005. (C) IL-4 production levels of basophils (50,000 cells/well) and Tfh (50,000 cells/well) at the indicated time points following stimulation with anti-IgE or CD3/28 beads. Data are shown as the mean \pm SEM. Statistical significances are determined by Student t-test, *p<0.05, **p<0.005, ***p<0.0005. Tfh, follicular helper T cell; TGF- β 1, transforming growth factor- β 1; SEM, standard error of the mean; ANOVA, analysis of variance; ns, no significance.

Figure 5

Examination of basophil homing into SLE patients' lymph nodes. (A) CD62L and CXCR4 expression of basophils obtained from healthy subjects. One representative FACS plot and cumulative mean FI are

shown. Data are shown as the mean \pm SEM. Statistical significances are determined by Student t-test. (B) Immunohistochemical analysis of basophils in the lymph node of an SLE patient using mouse anti-basophil antibodies. Scale bar, 50 μ m. mean FI, mean fluorescent intensity; SEM, standard error of the mean; SLE, systemic lupus erythematosus.

Figure 6

Cross-linking dsDNA and anti-dsDNA IgE increases IL-4 production in basophils from SLE patients. (A) IL4 expression levels in response to dsDNA in basophils obtained from healthy controls (HC) (n = 12), anti-dsDNA IgE negative SLE patients (n = 16), and anti-dsDNA IgE positive SLE patients (n = 11). (B) IL4 expression data from SLE patients were stratified into HC (n = 12), IgG⁺IgE⁺ (n = 10), and IgG⁺IgE⁻ (n = 4) according to the presence of anti-dsDNA IgG. Data are shown as the median (interquartile range). Statistical significances are determined by Kruskal-Wallis test followed by Steel's post-hoc test. dsDNA, double-stranded DNA; SLE, systemic lupus erythematosus.

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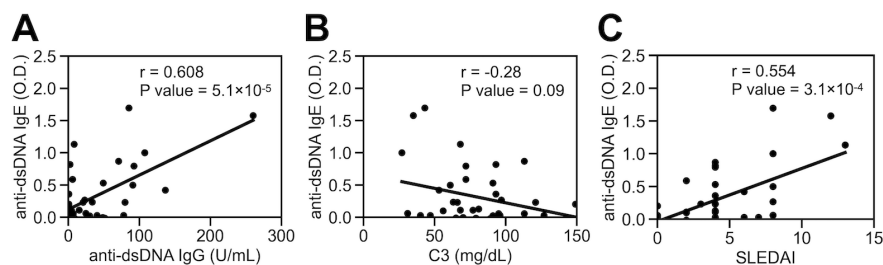


Figure 1_Sho et al.

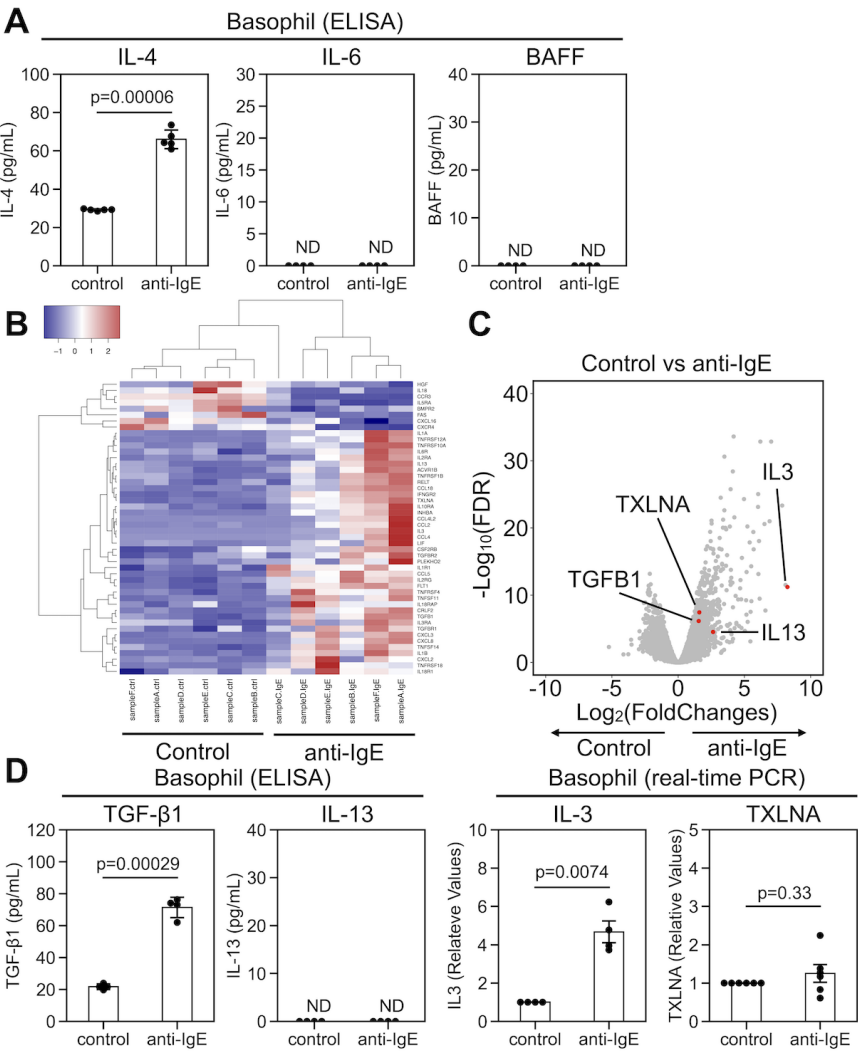


Figure 2_Sho et al.

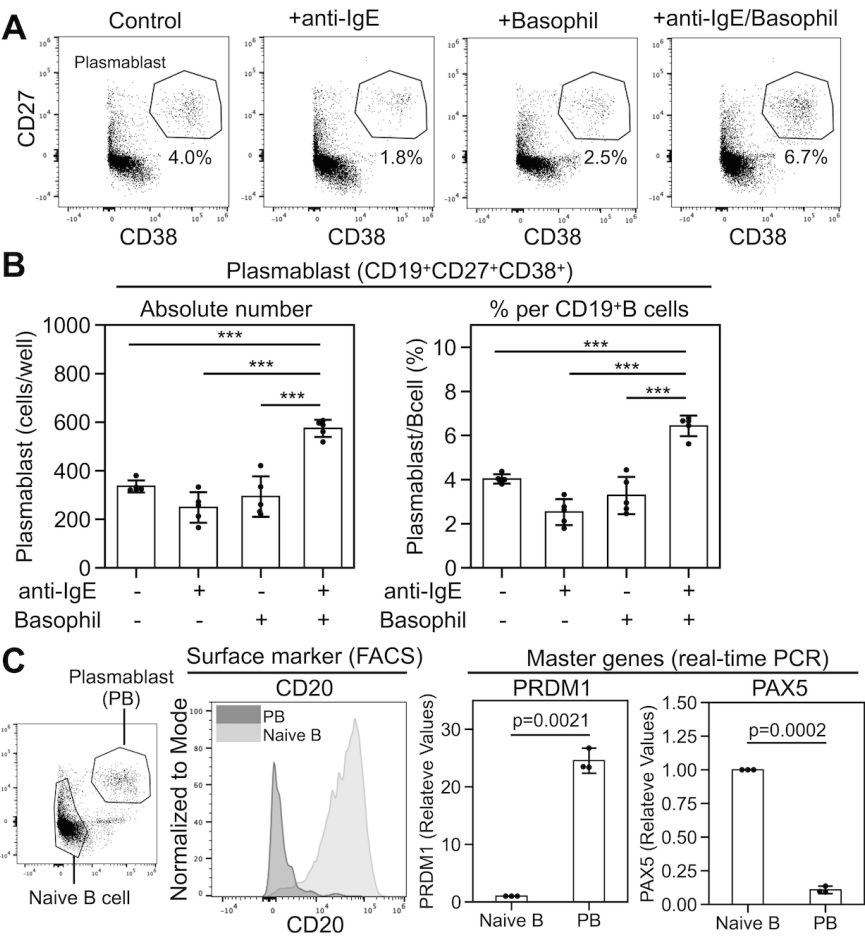


Figure 3_Sho et al.

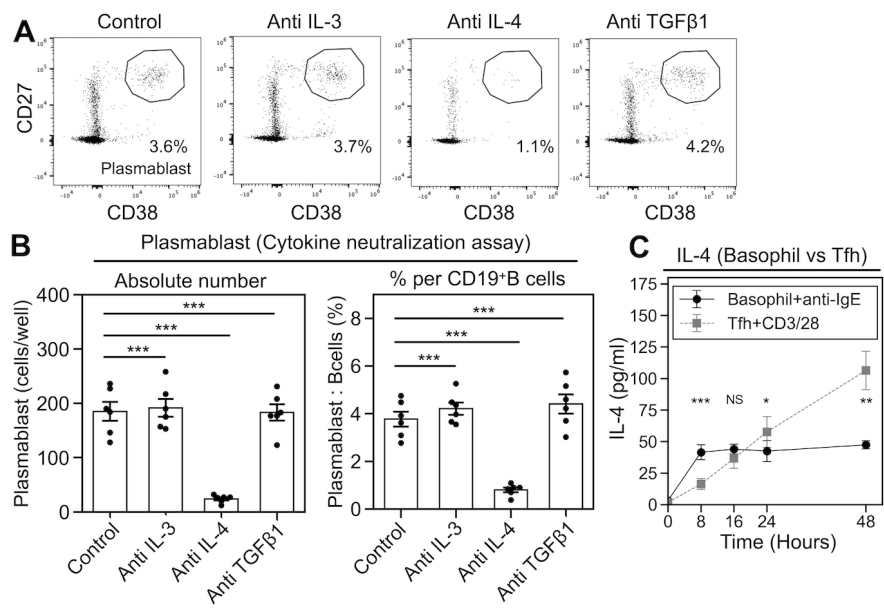


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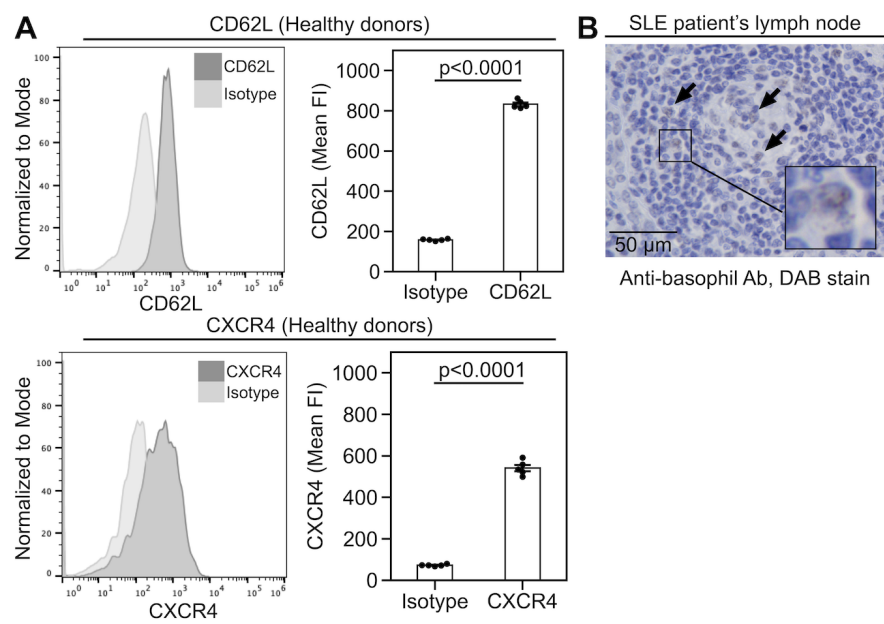


Figure 5_Sho et al.

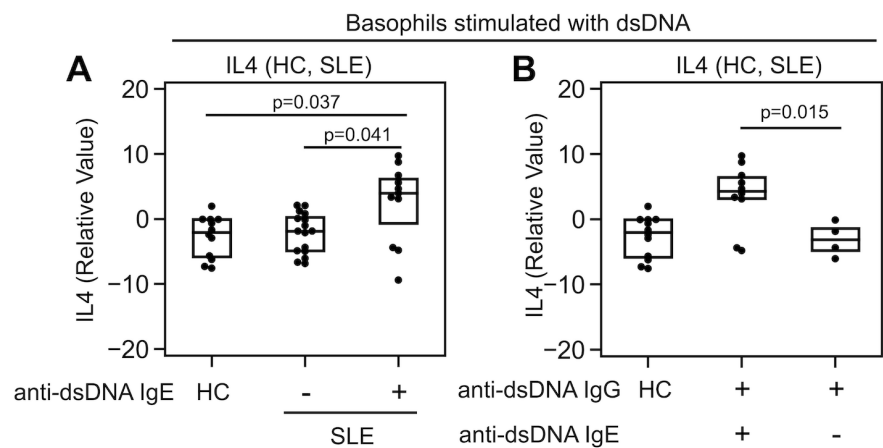


Figure 6_Shō et al.