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# Distinct Expression and Function of FcεRII in Human B Cells and Monocytes

Wenming Peng,\* William Grobe,\* Gisela Walgenbach-Brünagel,<sup>†</sup> Sabine Flicker,<sup>‡</sup> Chunfeng Yu,\* Marc Sylvester,<sup>§</sup> Jean-Pierre Allam,\* Johannes Oldenburg,<sup>¶</sup> Natalio Garbi,<sup>||</sup> Rudolf Valenta,<sup>‡</sup> and Natalija Novak\*

FcεRII is a multifunctional low-affinity IgER that is involved in the pathogenesis of allergic, inflammatory, and neoplastic diseases. Although discrepancies in FcεRII-mediated functions are being increasingly recognized, the consequences of FcεRII activation are not completely understood. In this study, we evaluated the expression of FcεRII on human blood cells and found that it was primarily expressed on monocytes and B cells. Although IL-4 promoted expression of the FcεRIIb isoform on B cells and monocytes, the expression of the FcεRIIa isoform was not dependent on IL-4. Furthermore, FcεRII predominantly bound allergen-IgE complexes on B cells but not on monocytes. FcεRII-mediated allergen-IgE complex uptake by B cells directed Ags to MHC class II-rich compartments. FcεRII-bearing monocytes and B cells expressed high levels of the FcεRII sheddase a disintegrin and metalloproteinase 10, which implies that they are important sources of soluble FcεRII. Moreover, we identified that IgE immune complex stimulation of FcεRII activated intracellular tyrosine phosphorylation via Syk in B cells but not in monocytes. Importantly, FcεRII-mediated signaling by allergen-IgE immune complexes increased IFN-γ production in B cells of allergic patients during the build-up phase of allergen-specific immunotherapy. Together, our results demonstrate that FcεRII mediates cell type-dependent function in allergic reactions. In addition, the results identify a novel allergen-IgE complex/FcεRII/Syk/IFN-γ pathway in allergic responses and suggest that FcεRII may play a role in regulating allergic reactions via modulating IFN-γ production in B cells. *The Journal of Immunology*, 2017, 198: 3033–3044.

Allergic diseases affect >20% of the population in industrialized countries and represent an increasing global public health problem (1, 2). Classic allergic immune responses are largely dependent on the interaction of IgE with IgERs (3). Binding of IgE to FcεRI and to FcεRII (or CD23) on human immune cells initiates and orchestrates allergic responses (3–5). Effects mediated by FcεRI have been extensively studied in animal models, human mast cells, basophils, monocytes, and dendritic cells (DCs) (2, 5, 6). In contrast, the consequences of FcεRII activation are still not completely understood.

As a C-type lectin receptor expressed by human B cells, follicular DCs, monocytes, and intestinal and respiratory epithelial

cells, FcεRII is thought to be critically involved in allergic and inflammatory diseases by regulating IgE synthesis, transport of allergens across gut and airway epithelial barriers, IgE-facilitated allergen presentation, and inflammatory responses (4, 7–11). Although FcεRII monomers bind IgE with low affinity, FcεRII is also capable of forming trimers that bind IgE with higher affinity (12). Yet the stability of the FcεRII trimer is temperature sensitive, which causes FcεRII to bind IgE more efficiently at lower temperatures (4–20°C) than at a higher temperature (37°C) (13, 14). Furthermore, a disintegrin and metalloproteinase 10 (ADAM10) cleaves membrane-bound FcεRII and releases various forms of soluble FcεRII (sFcεRII) (15, 16), which further interact with CD21 on the membrane of B cells to regulate IgE production (17–19). In humans, FcεRII exists in two isoforms, FcεRIIa and FcεRIIb, which differ only in their cytoplasmic N-terminal domains. FcεRIIa is expressed on B cells and is involved in endocytosis of IgE-binding Ags, whereas expression of FcεRIIb is inducible by IL-4 on different cell types, including B cells, monocytes, DCs, and epithelial cells (2, 4). FcεRII mediates different signals in various types of immune cells. Activated FcεRII induces the generation of cAMP (20), calcium flux, and polyphosphoinositide hydrolysis (21) in human B cells, and it mediates growth inhibition of leukemic B cells (22), whereas activated FcεRII triggers NO production and NF-κB activation in human monocytes (23, 24). However, the mechanisms by which FcεRII mediates different signals in human monocytes and B cells in allergic responses have not been elucidated in detail. In addition, unlike FcεRI, which contains ITAM in its cytoplasmic portion and mediates phosphorylation of signaling molecules after cross-linking of FcεRI (5), FcεRIIa and FcεRIIb have very short cytoplasmic tails that contain only 6 or 7 aa (4). Thus, it is very likely that FcεRII needs to use adaptors to mediate intracellular signaling events, such as protein phosphorylation.

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Abbreviations used in this article: ADAM10, a disintegrin and metalloproteinase 10; Bet v 1-FITC, FITC-conjugated birch pollen allergen Bet v 1; DC, dendritic cell; MHC-II, MHC class II; MS, mass spectrometry; NP, 4-hydroxy-3-nitrophenylacetyl hapten; NP-BSA, NP-conjugated BSA; sFcεRII, soluble FcεRII.

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Elevated FcεRII on peripheral lymphocytes was observed in patients with allergic diseases (25–27). Modified functions of FcεRII in allergic diseases were shown in human and animal models (28–30). In humans, allergen-specific tolerance can be induced by repetitive and continuous exposure of allergic patients to high amounts of the allergen to which they are allergic during allergen-specific immunotherapy (31). Therefore, allergen-specific immunotherapy may serve as a good model to investigate the functions of IgERs, including FcεRII, in response to allergen stimulation in humans.

It was the aim of our study to investigate cell type-dependent functions of FcεRII on human peripheral blood cells, such as B cells and monocytes, after stimulation of its natural ligands. FcεRII exerted different signaling in B cells and monocytes after stimulation by IgE immune complexes, mirrored by different levels of tyrosine phosphorylation. Proteomic analysis identified Syk as an adaptor molecule of FcεRII signaling in B cells. Furthermore, FcεRII-mediated signaling by allergen-IgE immune complexes increased IFN-γ production in B cells. Our results provide important evidence that FcεRII-mediated cell type-dependent functions may have a pivotal role in modulating allergic responses.

## Materials and Methods

### Study approval

Patients with hymenoptera venom allergy under venom immunotherapy ( $n = 18$ ; 7 men and 11 women; mean age,  $50.7 \pm 12.9$  y; age range, 25–75 y) from the Department of Dermatology and Allergy, University of Bonn were included in the study after giving their informed consent. The protocol was approved by the local ethics committee, and therapy was administered according to international guidelines (32–34).

### Detection of specific IgE

Blood samples from patients with hymenoptera venom allergy undergoing venom immunotherapy were taken before, as well as 2 d and 1 wk after, the start of immunotherapy. Serum-specific IgE against hymenoptera venom allergen (Ves v 5 or Api m 1) was detected by Phadia 250 immunoassay analyzers (Thermo Fisher Scientific, Uppsala, Sweden).

### Reagents

FITC-conjugated birch pollen allergen Bet v 1 (Bet v 1-FITC) and chimeric Bet v 1-specific IgE were described previously (35). Natural Bet v 1 was from Indoor Biotechnologies (Charlottesville, VA). Human FcR blocking reagent, PE-conjugated mouse anti-CD19 Ab (clone LT19), FITC-conjugated mouse anti-human CD34 Ab (clone AC136), protein G MicroBeads, and human CD19 and CD14 MicroBeads were from Miltenyi Biotec (Bergisch Gladbach, Germany). FITC-conjugated mouse anti-human CD4 Ab (clone RPA-T4), allophycocyanin-conjugated mouse anti-human CD14 Ab (clone M5E2), FITC-conjugated mouse anti-human CD14 Ab (clone M5E2), FITC-conjugated mouse anti-human CD103 Ab (clone Ber-ACT8), FITC-conjugated mouse anti-human CD19 Ab (clone HIB19), monoclonal mouse anti-human CD23 Ab (clone M-L233), PerCP-Cy5.5-conjugated mouse anti-human CD23 Ab (clone M-L233), PE-conjugated mouse anti-human Syk (pY348) Ab (clone I120-722), PE-conjugated rat anti-human IL-6 Ab (clone MQ2-6A3), PE-conjugated rat anti-human IL-10 Ab (clone JES3-19F1), and PE-conjugated mouse anti-human IFN-γ Ab (clone 4S.B3) were from BD Biosciences (Heidelberg, Germany). Rabbit polyclonal Ab against GRB2, goat polyclonal Ab against CD23, mouse mAb against GAPDH, mouse monoclonal anti-Syk Ab (clone 4D10), and protein G plus agarose were from Santa Cruz Biotechnology (Santa Cruz, CA). Mouse anti-phosphotyrosine mAb (clone 4G10) was from Millipore (Schwalbach, Germany). Human chimeric IgE anti 4-hydroxy-3-nitrophenylacetyl hapten (NP) and NP-conjugated BSA (NP-BSA) (ratio > 20) were from AbD Serotec (Puchheim, Germany). Monoclonal mouse anti-human CD23/FcεRII Ab (clone 138628) used for blockage of FcεRII was from R&D Systems (Wiesbaden-Nordenstadt, Germany). The Human CD23 ELISA Kit and monoclonal mouse anti-human HLA DR+DP+DQ Ab (clone CR3/43) were from Abcam (Cambridge, MA). PE-labeled mouse anti-human CD23 Ab (clone EBVC52) and PE-labeled mouse anti-human FcεRIα Ab (clone AER-37) were from eBioscience (San Diego, CA). Pacific blue-conjugated mouse anti-human

HLA-DR Ab (clone LN3) was from BioLegend (San Diego, CA). Polyclonal rabbit anti-human ADAM10 Ab was from New England Biolabs (Frankfurt, Germany). FITC-conjugated goat anti-mouse Ab was from Jackson ImmunoResearch (West Grove, PA). Normal mouse serum for blocking purposes was obtained from Dianova (Hamburg, Germany). Bee and yellow jacket venom were from BÜHLMANN Laboratories (Schönenbuch, Switzerland). All other reagents were obtained from Sigma-Aldrich (Taufkirchen, Germany), unless otherwise indicated.

### Isolation of CD14<sup>+</sup> and CD19<sup>+</sup> cells from human peripheral blood

PBMCs were isolated from blood of patients with hymenoptera venom allergy or from buffy coats of healthy blood donors (obtained from the blood bank of the University of Bonn) by Lymphoprep gradient technique, as described in the manufacturer's protocol (Axis Shield, Oslo, Norway). After blocking Fc receptor (cat. no. 130-059-901; Miltenyi Biotec), CD14<sup>+</sup> and CD19<sup>+</sup> cells were isolated from PBMCs by immunomagnetic selection with mAb against human CD14 and CD19 (Miltenyi Biotec), according to the manufacturer's instructions. The purity of CD14<sup>+</sup> and CD19<sup>+</sup> cells was >90%, as measured by flow cytometric staining. Cells were cultured in very low endotoxin medium RPMI 1640 (Biochrom, Berlin, Germany) with 1% antibiotic and antimycotics and 10% inactivated FCS. In some experiments, PBMCs, isolated monocytes, and B cells were cultured in culture medium with 500 U/ml rIL-4 (Gentaur, Aachen, Germany).

### Generation of IgE immune complexes

BSA-NP-IgE immune complexes were generated by incubation of anti-NP-IgE (0.7 μg/ml) with NP-BSA (10 μg/ml) in culture medium at room temperature for 1 h. Bet v 1-IgE immune complexes were generated by incubation of Bet v 1-FITC or natural Bet v 1 (2.5 μg/ml), together with IgE against Bet v 1 (5 μg/ml), in culture medium at room temperature for 1 h. The venom-IgE immune complexes were generated by incubation of yellow jacket or bee venom (11.5 ng/ml) with 10 μl/ml serum from patients with hymenoptera venom allergy (serum with specific IgE against rVes v 5 [30.8 kU/l] or specific IgE against Api m 1 [5.43 kU/l]) in culture medium at room temperature for 1 h.

### Flow cytometry

Flow cytometry was performed as described previously (36). For intracellular cytokine staining, cells were maintained in culture medium containing 0.1% GolgiPlug and GolgiStop (BD Biosciences) at 37°C for 3 h. Subsequently, cells were subjected to flow cytometric analysis. Isotype-matched mouse or rat IgG was used as Ab control. Cells were measured with a FACSCanto (BD Biosciences) and analyzed by FACSDiva (BD Biosciences) and FlowJo (TreeStar, Ashland, OR) software.

### RNA isolation, reverse transcription, and TaqMan real-time PCR

Total RNA was isolated using a NucleoSpin RNA II or XS kit (MACHEREY-NAGEL, Düren, Germany), including digestion of genomic DNA, and was subjected to cDNA synthesis with TaqMan RT reagents with random hexamers, according to the manufacturer's instructions (Applied Biosystems, Darmstadt, Germany). Fifty nanograms of total RNA was used for reverse transcription in a 20-μl reaction volume. The prepared cDNA was amplified with the help of TaqMan Gene Expression Master Mix and predesigned TaqMan Gene Expression Assays, according to the recommendations of the manufacturer, on an ABI Prism 7300 Sequence Detection System (all from Applied Biosystems). The following primers including probes were used: IL-6 (Hs99999032\_m1), IL-10 (Hs00174086\_m1), IFN-γ (Hs00989291\_m1), and 18s rRNA endogenous control (4310893E) (Applied Biosystems). All assays were performed according to the manufacturer's instructions. Relative quantification and calculation of the range of confidence were performed using the comparative ΔΔCT method (37).

### RT-PCR

To analyze the mRNA expression of FcεRIIa, FcεRIIb, and ADAM10 in human B cells and monocytes, primers were synthesized (Microsynth, Balgach, Switzerland), and PCRs were performed using the following primers, as described previously (8, 38): ADAM10\_forward: 5'-TCC ACA GCC CAT TCA GCA A-3', ADAM10\_reverse: 5'-AGG CAC TAG GAA GAA CCA A-3'; FcεRIIa\_forward: 5'-ATG GAG GAA GGT CAA TAT TC-3', FcεRIIa\_reverse: 5'-TCC AGC TGT TTT AGA CTC TG-3'; and FcεRIIb\_forward: 5'-ATG AAT CCT CCA AGC CAG-3', FcεRIIb\_reverse: 5'-CAC AGG AGA AGC AGA GTC AG-3'. The amount of human template cDNA in different PCRs was determined with a parallel

PCR for a 244-bp fragment of the GAPDH housekeeping gene with the following primers: GAPDH\_forward: 5'-CCA CAT CGC TCA GAC ACC AT-3' and GAPDH\_reverse: 5'-GGC AAC AAT ATC CAC TTT ACC AGA GT-3'. PCR products were resolved on 1.3% agarose gels and visualized by ethidium bromide staining.

#### *SYBR Green-based real-time PCR assay*

The SYBR Green-based real-time PCR assays were performed to quantitatively determine the mRNA expression of FcεRIIa and FcεRIIb in human monocytes and B cells using SYBR Green Supermix (Bio-Rad, Munich, Germany). The assay was performed in a 20-μl reaction mixture containing 10 μl of 2× SYBR Green Supermix, 1 μM each primer, and 8 μl of 10-fold diluted cDNA template. The thermal cycling conditions were as follows: an initial denaturation step at 95°C for 5 min, 35 cycles of PCR amplification at 95°C for 15 s, 54°C for 20 s, and 60°C for 40 s, followed by a melting curve analysis program, according to the recommendations of the manufacturer, on an ABI Prism 7300 Sequence Detection System (Applied Biosystems).

#### *Mass spectrometry*

To identify putative adapter molecules of activated FcεRII, we performed proteomic analysis with mass spectrometry (MS). Briefly, freshly isolated human PBMCs from buffy coats were suspended in RPMI 1640 medium containing 2% FCS and cultured in cell culture flasks (20 ml of cell suspension per 75-cm<sup>2</sup> flask) at 37°C for 1 h. After incubation, adherent cells were washed twice with ice-cold PBS and cultured in medium containing 500 U/ml IL-4 in a 37°C, 5% CO<sub>2</sub> incubator for 2 d to increase surface FcεRII expression. After 2 d of culture, cells were collected and stimulated with BSA-NP-IgE immune complexes at 37°C for 1 h. After two rinses with ice-cold PBS, ~80 × 10<sup>6</sup> cells were lysed in ice-cold lysis buffer (1% Triton X-100 in PBS with 1 mM PMSF, 5 μg/ml aprotinin, and 5 μg/ml leupeptin) by passing several times through a 25-gauge needle attached to a 1-ml syringe. After 15 min of extraction on ice, insoluble material was removed by centrifugation and the supernatant was collected. The supernatant was immunoprecipitated by incubating with 2 μg/ml of either mouse IgG isotype control or mouse mAb anti-FcεRII (clone M-L233) for 2 h, and then with protein G MicroBeads overnight at 4°C with rotation. The beads were washed 2 times with cold lysis buffer, and bound proteins were eluted with 0.1 M glycine (pH 2.7). Eluted proteins were separated by one-dimensional or two-dimensional SDS-PAGE electrophoresis. Proteins were visualized by silver staining using a Pierce Silver Stain Kit (Thermo Fisher Scientific, Waltham, MA). After comparison of silver staining of protein bands precipitated by IgG isotype control and anti-FcεRII Abs, bands of anti-FcεRII Ab-precipitated protein were cut and sent to the Institute of Biochemistry and Molecular Biology, University of Bonn for MS detection. MS results were analyzed with the protein-protein interaction tool STRING 10 (<http://string-db.org/>) and the DAVID gene functional classification tool (<http://david.abcc.ncifcrf.gov/>) (39).

#### *Immunoprecipitation and immunoblotting*

To detect cellular signaling adaptors that bind to FcεRII after activation, FcεRII complexes were immunoprecipitated, separated by SDS-PAGE, and transferred to a polyvinylidene fluoride membrane (Millipore, Billerica, MA). Blots were blocked with 5% nonfat milk in TBS (20 mM Tris [pH 7.5] and 0.15 M NaCl) containing 0.1% Tween 20 for 1 h at room temperature and incubated with 1:1000 diluted primary Ab overnight at 4°C, followed by 1:2000 diluted HRP-conjugated secondary Abs (Santa Cruz Biotechnology). Proteins were detected using an ECL Western blot detection system (Amersham Biosciences, Freiburg, Germany).

#### *Immunofluorescence staining*

Immunofluorescence staining was performed as described elsewhere (40). Slides were analyzed with a confocal laser scanning microscope (Leica TCS SP8) using Leica LAS AF software (both from Leica Mikrosysteme Vertrieb, Wetzlar, Germany) and ImageJ software (National Institutes of Health) for documentation and analysis.

#### *Statistical analysis*

Statistical analysis was performed with GraphPad Prism 5 (GraphPad, La Jolla, CA) or SPSS 22 for Windows (SPSS, Chicago, IL). Data distribution was examined using the Shapiro-Wilk normality test. Normally distributed data are shown as mean ± SEM. Nonnormally distributed data are shown as median ± interquartile range. Quantitative values were compared among the groups using one-way repeated-measures ANOVA (Tukey multiple-comparisons test) or a paired Student *t* test for normally distributed data. A Wilcoxon matched-

pairs test was used for analysis of nonnormally distributed data. The statistical test used and *p* values are indicated in each figure legend. Any *p* values are two-sided and subject to a global significance level of 5%.

## **Results**

### *B cells are the major FcεRII-expressing cells in human peripheral blood*

We showed that the vast majority of human peripheral FcεRII-expressing cells did not express FcεRI (Fig. 1A). To evaluate the expression of FcεRII in various types of human leukocytes, human peripheral blood cells were double stained with Abs against lineage markers, such as CD1c (DCs), CD4 and CD8 (T cells), CD14 (monocytes), CD34 and CD103 (stem cells), and CD19 (B cells), as well as with an Ab against FcεRII. Flow cytometry results demonstrated that human CD14<sup>+</sup> monocytes and CD19<sup>+</sup> B cells express FcεRII (Fig. 1B). The percentage and number of CD19<sup>+</sup> B cells expressing FcεRII were ~20- and 10-fold higher than those of FcεRII-expressing CD14<sup>+</sup> monocytes (Fig. 1C). Although very few CD1c<sup>+</sup>CD19<sup>−</sup> myeloid DCs expressed FcεRII, the total number of FcεRII<sup>+</sup> DCs in peripheral blood was negligible compared with monocytes and B cells (Fig. 1B, 1C).

### *FcεRIIa and FcεRIIb are differentially expressed by human monocytes and B cells*

We next compared the expression profiles of FcεRII on human monocytes and B cells constitutively and after activation with IL-4, which is known to induce FcεRII expression (4). Surface expression of FcεRII on monocytes and B cells at 0, 24, and 48 h after IL-4 stimulation was evaluated. We showed that B cells express FcεRII constitutively (Fig. 2A). Although IL-4 induced FcεRII expression on monocytes and B cells, human monocytes expressed significantly higher levels of FcεRII than B cells after incubation with IL-4 (Fig. 2B).

To further investigate the expression of FcεRII isoforms on human monocytes and B cells, monocytes and B cells were collected before and after IL-4 stimulation and subjected to FcεRIIa and FcεRIIb PCR analysis. B cells constitutively expressed mRNA of the FcεRIIa isoform (Fig. 2C). Results of SYBR Green-based quantitative real-time PCR demonstrated that IL-4 did not increase the expression of FcεRIIa on human B cells (Fig. 2D). However, freshly isolated human monocytes and B cells weakly expressed FcεRIIb mRNA, which was further increased by IL-4 stimulation on both cell types (Fig. 2C, 2D).

### *B cells predominantly bind IgE immune complexes via FcεRII*

To investigate the binding of its natural ligands to FcεRII on human B cells and monocytes, we incubated Bet v 1-FITC, the major birch pollen allergen, as well as FITC-conjugated Bet v 1-IgE immune complexes, with IL-4-stimulated monocytes and B cells. After 24 and 48 h of incubation, monocytes efficiently took up Bet v 1 and Bet v 1-IgE immune complexes; however, blockage of FcεRII did not reduce the uptake of Bet v 1-IgE complexes by monocytes (Fig. 3). In contrast, B cells showed a high capacity to bind Bet v 1-IgE complexes compared with Bet v 1 (Fig. 3). Furthermore, blockage of FcεRII significantly decreased the uptake of Bet v 1-IgE immune complexes by B cells (Fig. 3).

Moreover, we used FITC-conjugated dextran as an Ag control to investigate whether the formation of an IgE immune complex is important for FcεRII-mediated binding and uptake of allergen on B cells. We showed that B cells did not increase binding and uptake of FITC-conjugated dextran when incubated with the mixture prepared by preincubation of FITC-conjugated dextran with the IgE specifically against Bet v 1 compared with FITC-conjugated dextran alone. However, B cells still efficiently took up Bet v 1-FITC/IgE

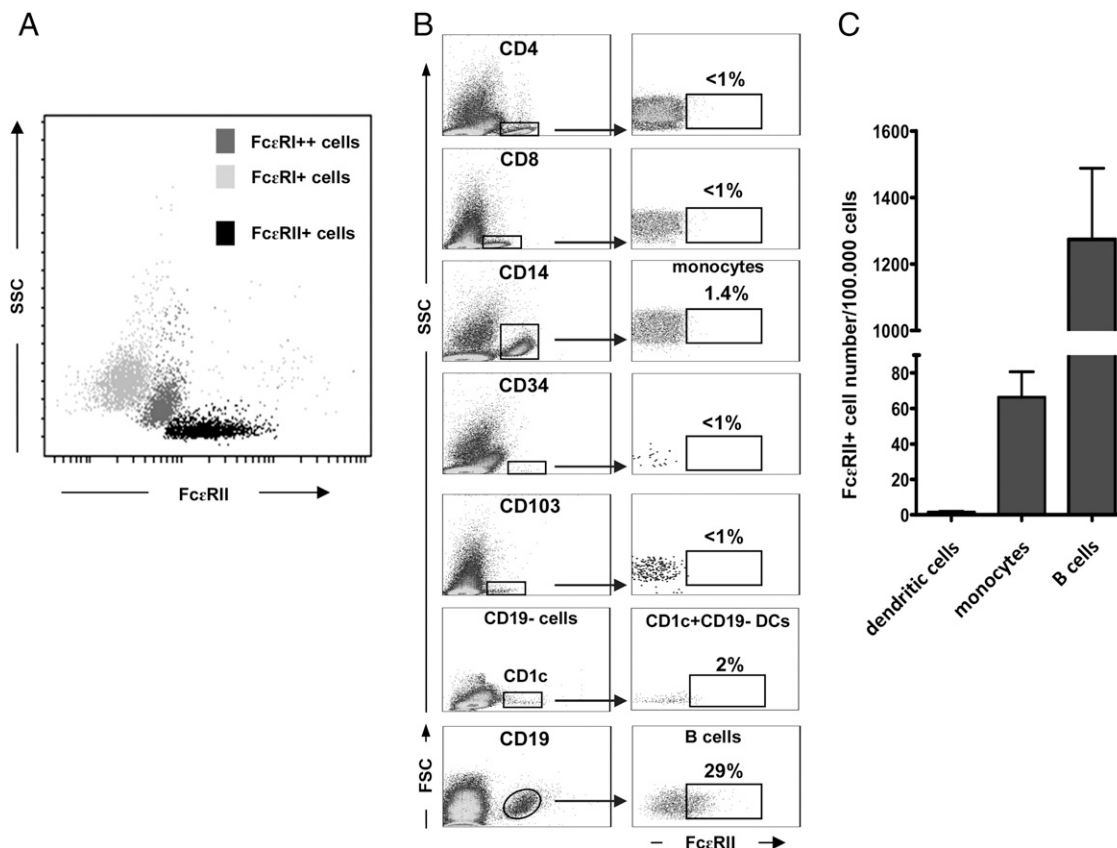


**FIGURE 1.** Expression of FcεRII on human peripheral blood cells. **(A)** Different expression of FcεRI and FcεRII on human peripheral blood cells. **(B)** Expression of FcεRII on CD4<sup>+</sup>, CD8<sup>+</sup>, CD14<sup>+</sup>, CD34<sup>+</sup>, CD103<sup>+</sup>, CD19<sup>+</sup>CD1c<sup>+</sup>, and CD19<sup>+</sup> cells. **(C)** Number of FcεRII<sup>+</sup> DCs ( $n = 4$  donors), monocytes ( $n = 8$  donors), and B cells ( $n = 8$  donors) per 100,000 cells. In (A) and (B), one representative experiment out of  $n = 3$  is shown.

immune complexes prepared by preincubation of Bet v 1–FITC with the specific IgE against Bet v 1 (Supplemental Fig. 1).

#### *Allergen complexes captured by FcεRII are directed to MHC class II–rich compartments in B cells*

In line with a previous study (41), our immunoblotting results demonstrated that MHC class II (MHC-II) molecules and FcεRII form complexes after BSA–NP–IgE immune complex stimulation (Fig. 4A).

To investigate the interaction of FcεRII with MHC-II molecules after allergen–IgE immune complex stimulation, we used Bet v 1–FITC in triple immunofluorescence staining, together with Abs against MHC-II molecules and FcεRII. Enlarged FcεRII–MHC-II–Bet v 1 structures were observed in B cells after stimulation with Bet v 1–IgE immune complexes compared with B cells stimulated with Bet v 1 alone (Fig. 4B). Furthermore, semiquantitative analysis based on confocal laser scanning microscopy images demonstrated increased Bet v 1 binding when it formed immune complexes with IgE (Fig. 4C). These data demonstrate a close association of FcεRII with MHC-II in B cells after allergen–IgE immune complex stimulation.

#### *Activation of FcεRII by IgE immune complexes in human B cells, but not monocytes, induces tyrosine phosphorylation of downstream proteins*

Tyrosine phosphorylation represents an important mechanism of signal transduction in all eukaryotic cells (42). To investigate FcεRII-mediated signaling in B cells and monocytes, we incubated BSA–NP–IgE immune complexes with IL-4-stimulated human PBMCs. We showed that stimulation of BSA–IgE immune

complexes induced strong intracellular protein phosphorylation in FcεRII-expressing B cells but not in monocytes (Fig. 5A).

We next confirmed that IgE immune complexes are important for FcεRII-mediated tyrosine phosphorylation of downstream proteins of B cells. For this purpose, IL-4-stimulated human PBMCs were treated with anti-NP–IgE, NP–BSA, or BSA–NP–IgE immune complexes or were preincubated with anti-NP–IgE, washed, and cross-linked with NP–BSA. We showed that only stimulation of BSA–IgE immune complexes induced strong intracellular protein tyrosine phosphorylation in FcεRII-expressing B cells (Fig. 5B).

#### *FcεRII on human B cells uses Syk after IgE immune complex stimulation*

To investigate FcεRII-mediated signaling in human PBMCs, after IL-4 stimulation, molecules attached to IgE immune complex-activated FcεRII were precipitated and detected with MS. In addition to our results showing that IgE immune complexes induced strong tyrosine phosphorylation in human B cells, many molecules, such as integrins, MHC molecules, MAPK, Syk, and GRB2, were recruited to activated FcεRII (Fig. 6A).

Syk is an essential molecule for intracellular signal transduction in hematopoietic cells (43, 44), and GRB2 is important for B cell functions. Immunoblot results confirmed the MS data by showing that Syk and GRB2 were recruited to FcεRII activated by IgE immune complexes in human PBMCs (Fig. 6B). Furthermore, activated FcεRII by IgE immune complexes in B cells, but not monocytes, induced robust Syk phosphorylation (Fig. 6C, 6D). Together, we identified Syk as an important adaptor for FcεRII-mediated signaling in human B cells.

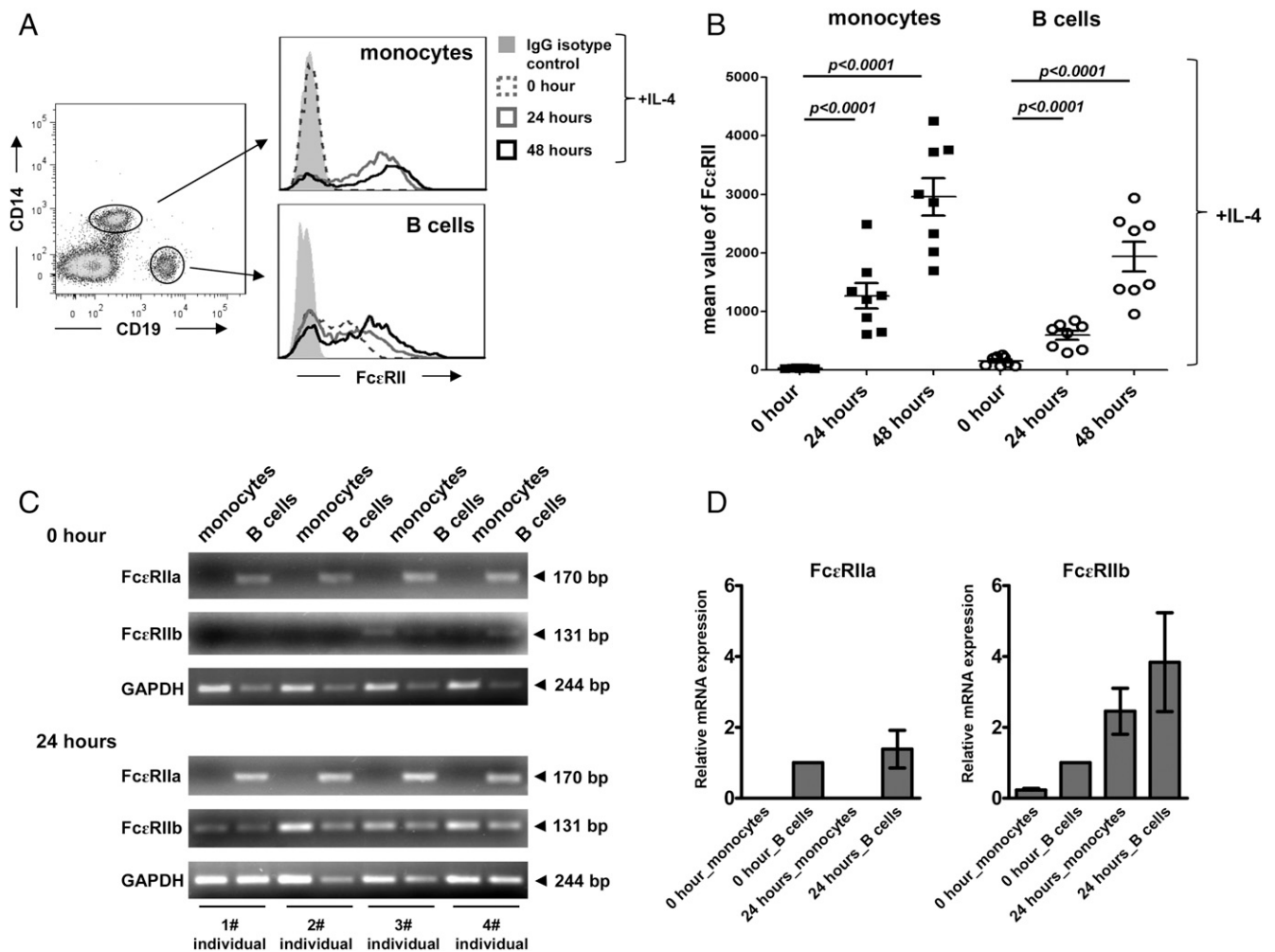


**FIGURE 2.** IL-4 induces different FcεRIIa and FcεRIIb expression in human monocytes and B cells. **(A)** Freshly isolated human PBMCs were cultured in medium containing IL-4. At 0, 24, and 48 h, PBMCs were collected and stained with the Ab mixture anti-CD14/anti-CD19/isotype-matched IgG or anti-CD14/anti-CD19/anti-FcεRII. One representative experiment of FcεRII expression on monocytes and B cells is shown ( $n = 8$  donors). **(B)** Mean values of FcεRII expression on monocytes and B cells are shown as scattered dot plots ( $n = 8$  donors, mean  $\pm$  SEM). The  $p$  values were calculated using a paired Student  $t$  test. **(C)** Freshly isolated monocytes and B cells from the same blood donor were cultured in medium containing IL-4. At 0 and 24 h, cells were collected and subjected to RT-PCR. Amplified PCR products of FcεRIIa, FcεRIIb, and GAPDH of IL-4-stimulated monocytes and B cells at 0 and 24 h. **(D)** Quantitative SYBR Green-based real-time PCR was performed with cDNA made from IL-4-stimulated B cells and monocytes at 0 and 24 h to analyze the expression levels of FcεRIIa and FcεRIIb. GAPDH was used as internal control. Fold change in mRNA expression over the expression of target gene in B cells at the time point 0 h is shown ( $n = 4$  donors, mean  $\pm$  SEM).

ADAM10 is a major FcεRII sheddase that releases sFcεRII and, thereby, plays an important role in allergic diseases (15, 16). In this study, we showed that FcεRII-expressing human monocytes and B cells expressed high levels of ADAM10, which implies that both are important sources of sFcεRII (Fig. 6E).

#### Activation of FcεRII on B cells by IgE immune complexes induces IFN- $\gamma$ production by B cells

In a previous study, we observed elevated IFN- $\gamma$  mRNA levels in PBMCs from patients during the early phase of hymenoptera venom immunotherapy (45). Recently, IFN- $\gamma$ -producing B cells were identified in mouse models (46–51). Thus, we investigated whether FcεRII-mediated signaling by IgE immune complexes resulted in the production of IFN- $\gamma$  by B cells.

As a first step, we investigated whether FcεRII-mediated signaling was involved in the IFN- $\gamma$  production by B cells in allergic patients during allergen stimulation. For this purpose, blood samples were taken from patients during the build-up phase of venom immunotherapy (Fig. 7A). After allergen injections, increased levels of

specific IgE against Ves v 5, one of the major allergens found in yellow jacket venom, were observed in the serum of allergic patients, reflecting activation of allergic immune responses (Fig. 7B). Furthermore, elevated surface expression of FcεRII (Fig. 7C) and serum levels of sFcεRII were observed in patients who underwent immunotherapy (Fig. 7D). mRNA expression of activating and suppressive factors in monocytes and B cells, such as IL-10, IFN- $\gamma$ , and IL-6, was evaluated by TaqMan probe-based real-time PCR. After allergen injections, transient expression of IL-10 and IFN- $\gamma$  mRNA was observed in monocytes, whereas increased expression of IFN- $\gamma$  mRNA was observed in B cells 2 d and 1 wk after the start of allergen treatment (Fig. 7E). Similar to real-time PCR results, elevated IL-10 and IFN- $\gamma$  protein expression was observed in monocytes and elevated IFN- $\gamma$  protein expression was observed in B cells after the onset of immunotherapy (Fig. 7F).

To investigate whether FcεRII-mediated signaling modulates IFN- $\gamma$  production in human B cells, PBMCs from patients with hymenoptera venom allergy were stimulated with venom or venom-IgE immune complexes, with or without previous FcεRII

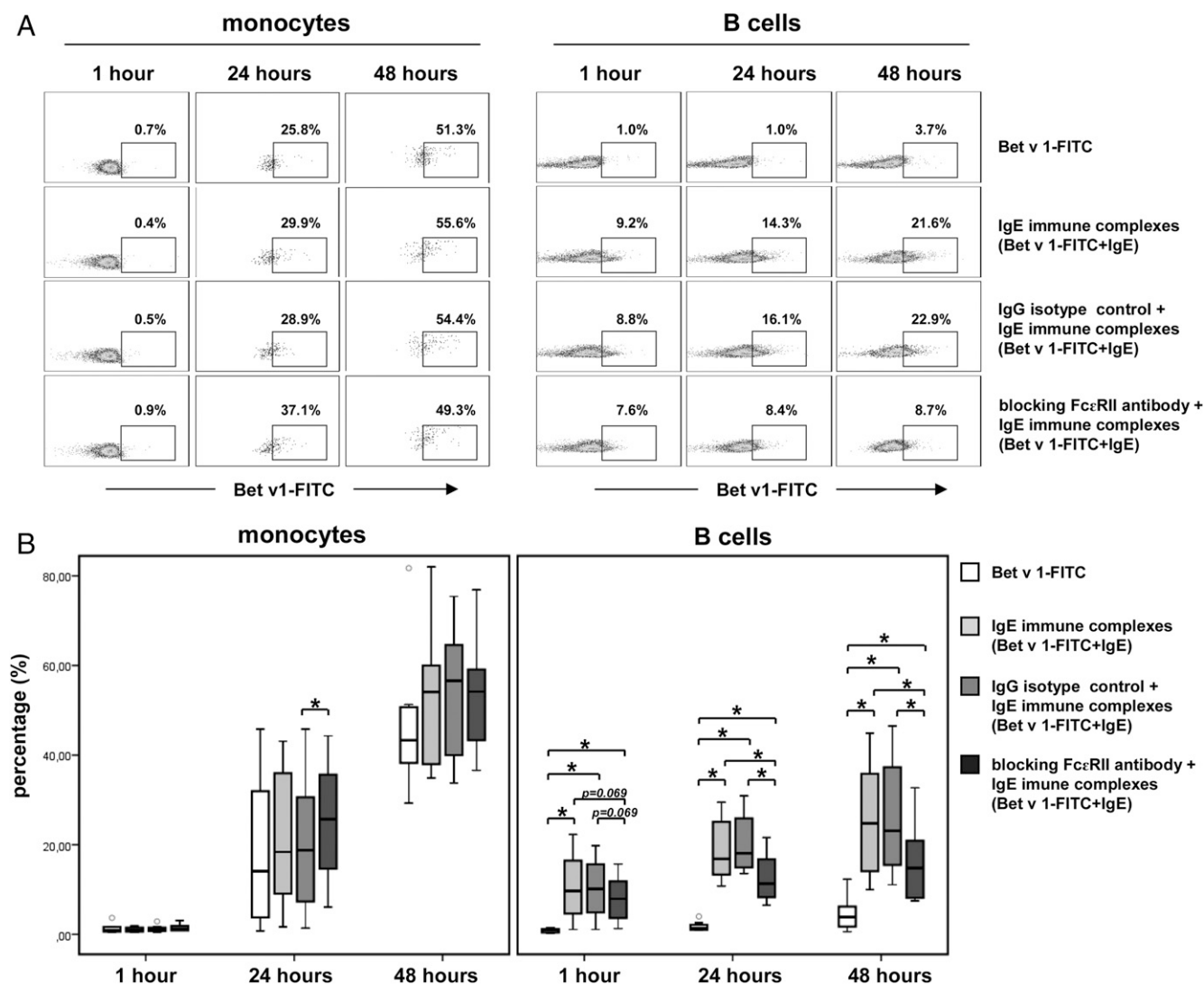


**FIGURE 3.** Human B cells bind and take up Bet v 1-IgE immune complexes via FcεRII. **(A)** Monocytes and B cells from the same blood donor were cultured with Bet v 1-FITC and Bet v 1-FITC/IgE immune complexes, with or without FcεRII blockage, in culture medium containing IL-4. One representative result of Bet v 1-FITC<sup>+</sup> monocytes and B cells at 0, 24, and 48 h from  $n = 8$  is shown. **(B)** Statistical analysis of the percentage of Bet v 1-FITC<sup>+</sup> monocytes and B cells at 0, 24, and 48 h ( $n = 8$  donors). \* $p < 0.05$ , Wilcoxon-test.

blockage. We showed that the percentage of IFN- $\gamma$ -producing B cells increased after stimulation with venom-IgE immune complexes compared with venom alone. Furthermore, FcεRII blockage decreased the percentage of IFN- $\gamma$ -producing B cells in response to venom-IgE immune complex stimulation (Fig. 7G).

Together, our results demonstrated that stimulation of FcεRII with allergen-IgE immune complexes plays a role in the induction of IFN- $\gamma$  production by B cells.

## Discussion

We demonstrated that FcεRII has distinct functions on human monocytes and B cells. We showed that human monocytes and B cells express different FcεRII isoforms. Furthermore, human B cells, but not monocytes, bind and take up allergen-IgE immune complexes through FcεRII, which associates with the MHC-II pathway. Moreover, allergen-IgE complex-stimulated FcεRII on human B cells, but not monocytes, uses Syk to activate tyrosine phosphorylation. In addition, FcεRII activates a small portion of IFN- $\gamma$ -producing B cells, which might be important for allergic immune responses after allergen stimulation.

Although FcεRII has been investigated for more than two decades, data with regard to FcεRII-mediated signal and function are not unanimous because studies were performed using various types and lines of cells at different temperatures or by activating cells with stimulatory anti-FcεRII Abs rather than with its natural ligand: IgE or allergen-IgE complexes. The outcome of FcεRII-mediated activation is affected by many factors. For example, the interaction of FcεRII with IgE is temperature sensitive (13, 14), and the expression of FcεRII is increased in allergic subjects by Th2-type cytokines (4). Furthermore, FcεRII is coexpressed on various immune cells together with other immune receptors that may contest allergen-IgE complex binding and uptake with FcεRII and, thereby, change the outcome of FcεRII-mediated activation. Therefore, we maintained human peripheral cells with the typical Th2 type cytokine IL-4 and stimulated them with different types of allergen-IgE complexes at normal body temperature (37°C) to better understand the physiological roles of FcεRII in very complicated allergic responses.

Elevated FcεRII was observed in patients with allergic diseases (25–27), as well as in patients with autoimmune diseases (52, 53) or B-chronic lymphocytic leukemia (22, 54, 55). The pathogenic

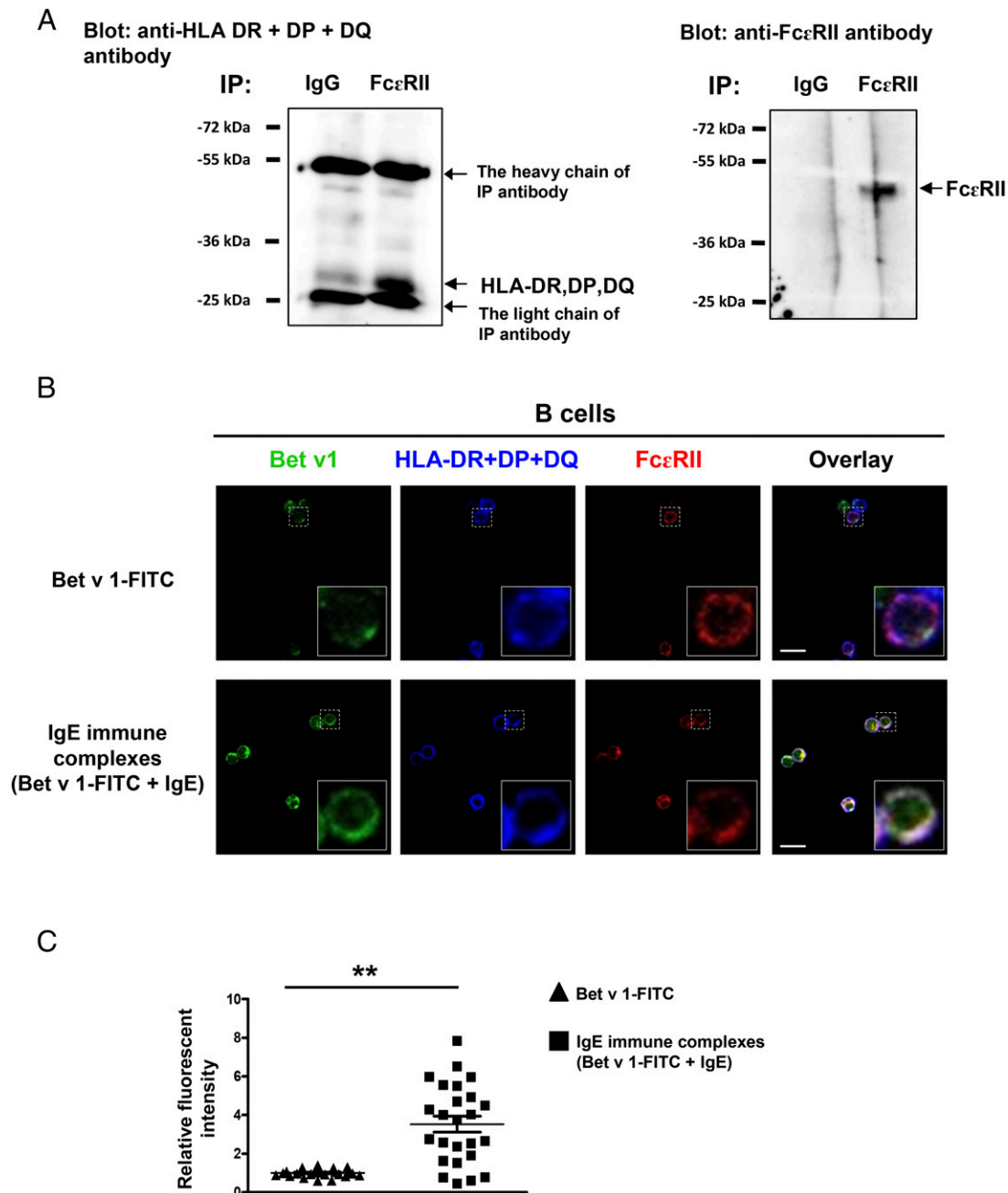


**FIGURE 4.** Allergen complexes captured by FcεRII are directed to MHC-II-rich compartments in B cells. **(A)** After 2 d of culture with IL-4, human PBMCs were stimulated with BSA-NP-IgE immune complexes for 1 h. Then, cell extracts were prepared as described in *Materials and Methods*. Coimmunoprecipitation experiments were performed with cell lysates prepared in the presence of mouse monoclonal anti-FcεRII Ab. The mouse IgG served as Ab isotype control. The precipitated proteins were resolved by SDS-PAGE and transferred to polyvinylidene difluoride membranes. Western blotting was performed using mouse mAbs against HLA-DR, DP, DQ. The cross-reactions of mouse IgG L chains and H chains were also labeled (left panel). After detection, the membrane was stripped and subjected to FcεRII immunoblotting using a polyclonal goat anti-FcεRII Ab (right panel). Results are representative of  $n = 3$  independent experiments. **(B)** Triple immunofluorescence confocal laser scanning microscopy of Bet v 1, FcεRII, and MHC-II molecules on human B cells. IL-4-stimulated human CD19<sup>+</sup> B cells were cultured with Bet v 1-FITC/IgE immune complexes, or Bet v 1-FITC (green) alone as control, at 37°C for 3 h. Immunofluorescence staining was performed with the help of PE-Cy5-conjugated anti-FcεRII (red) and Pacific blue-conjugated anti-HLA-DR (blue) Abs. One of three independent experiments is shown (scale bars, 10 μm). **(C)** Semiquantitative fluorescence analysis of Bet v 1 binding to B cells. FITC-fluorescence intensity of randomly selected single B cells was detected by confocal laser scanning microscopy. Results are shown as relative fluorescent intensity, reported as ratios of each cell intensity to the average fluorescence intensity of Bet v 1-FITC binding.  $**p < 0.005$ , Mann-Whitney  $U$  test.

functions of FcεRII were supported by the observations that administration of an anti-FcεRII Ab dose dependently reduced serum IgE concentrations in allergic patients (28) and ameliorated collagen-induced arthritis in mice (29). Furthermore, blockage of FcεRII relieved allergen-induced symptoms in the gastrointestinal tract in mouse models (30). Due to the structural differences in

their intracellular domains, FcεRIIa and FcεRIIb exert distinct functions and activate divergent signaling pathways (4, 7). Our data demonstrated that constitutive expression of FcεRIIa by B cells is not affected by IL-4 stimulation (Fig. 2D). In contrast, FcεRIIb is an inducible receptor that is expressed on various cell types, including monocytes, B cells, and respiratory and intestinal

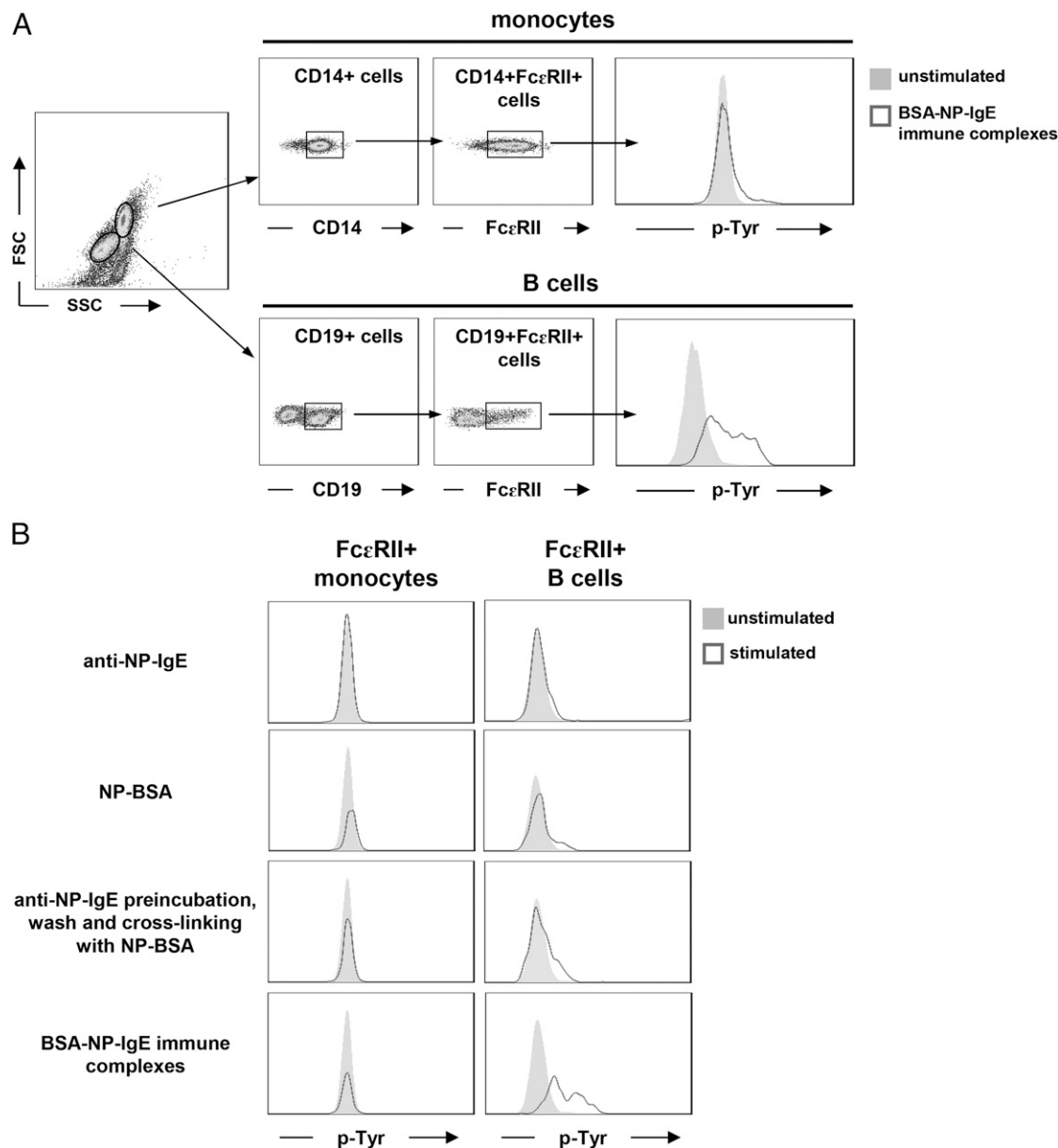


**FIGURE 5.** Stimulation of FcεRII on human B cells, but not monocytes, mediates elevated intracellular levels of phospho-tyrosine. Human PBMCs were cultured with IL-4 for 24 h to boost the expression of FcεRII. **(A)** Intracellular phospho-tyrosine levels of CD14<sup>+</sup>FcεRII<sup>+</sup> monocytes and CD19<sup>+</sup>FcεRII<sup>+</sup> B cells, with or without stimulation by IgE immune complexes. **(B)** Intracellular phospho-tyrosine levels of the cells stimulated with anti-NP-IgE, NP-BSA, or BSA-NP-IgE immune complexes or of the cells preincubated with anti-NP-IgE, washed, and cross-linked with NP-BSA, in FcεRII<sup>+</sup> monocytes and B cells. All results are representative of independent experiments from  $n = 3$  donors.

epithelial cells (4). It was shown that FcεRIIa binds Ag-IgE immune complexes more efficiently than does FcεRIIb (7). Our results support this observation by showing that blockage of FcεRII on B cells decreased endocytosis of Bet v 1-IgE immune complexes, whereas blockage of FcεRII on monocytes did not show this effect.

Previous studies showed that stimulation of FcεRII in human B cells led to the activation of ERK1/2, the tyrosine kinase Fyn, and the serine/threonine kinase Akt, whereas activation of FcεRII in the monocytic cell line U937 did not activate Fyn and Akt (56, 57). With the help of MS and immunoblotting, we demonstrated that FcεRII-mediated signaling by allergen-IgE complexes is cell type dependent. Although human B cells and monocytes expressed high levels of FcεRII after IL-4 stimulation, activated FcεRII on B cells, but not on monocytes, initiated strong phosphorylation of Syk (Fig. 6D). Syk is a fundamental signaling molecule that is expressed in a wide range of immune cells, in-

cluding B cells and monocytes. Syk interacts with ITAM or intracellular adaptors to initiate cell signaling. Syk is required for proliferation and differentiation of normal B cells (58, 59), whereas constitutive activation of Syk may transform normal B cells to malignancy. Overexpression of FcεRII is frequently observed in B-chronic lymphocytic leukemia, an adult leukemic disease (22). Moreover, it was shown that Ab-mediated cross-linking of FcεRII suppresses the growth of leukemic B cells (22, 54, 55). Because of the wide range of functions of Syk, activated FcεRII may use it to regulate cell proliferation and immune reactions. Which B cell-specific ITAM-containing protein is involved in FcεRII-mediated signaling remains under investigation.

Human monocytes are generated from hematopoietic stem cell precursors in the bone marrow and circulate in the bloodstream. Furthermore, monocytes are able to migrate from the bloodstream into tissues and to differentiate into tissue DCs based on the stimuli

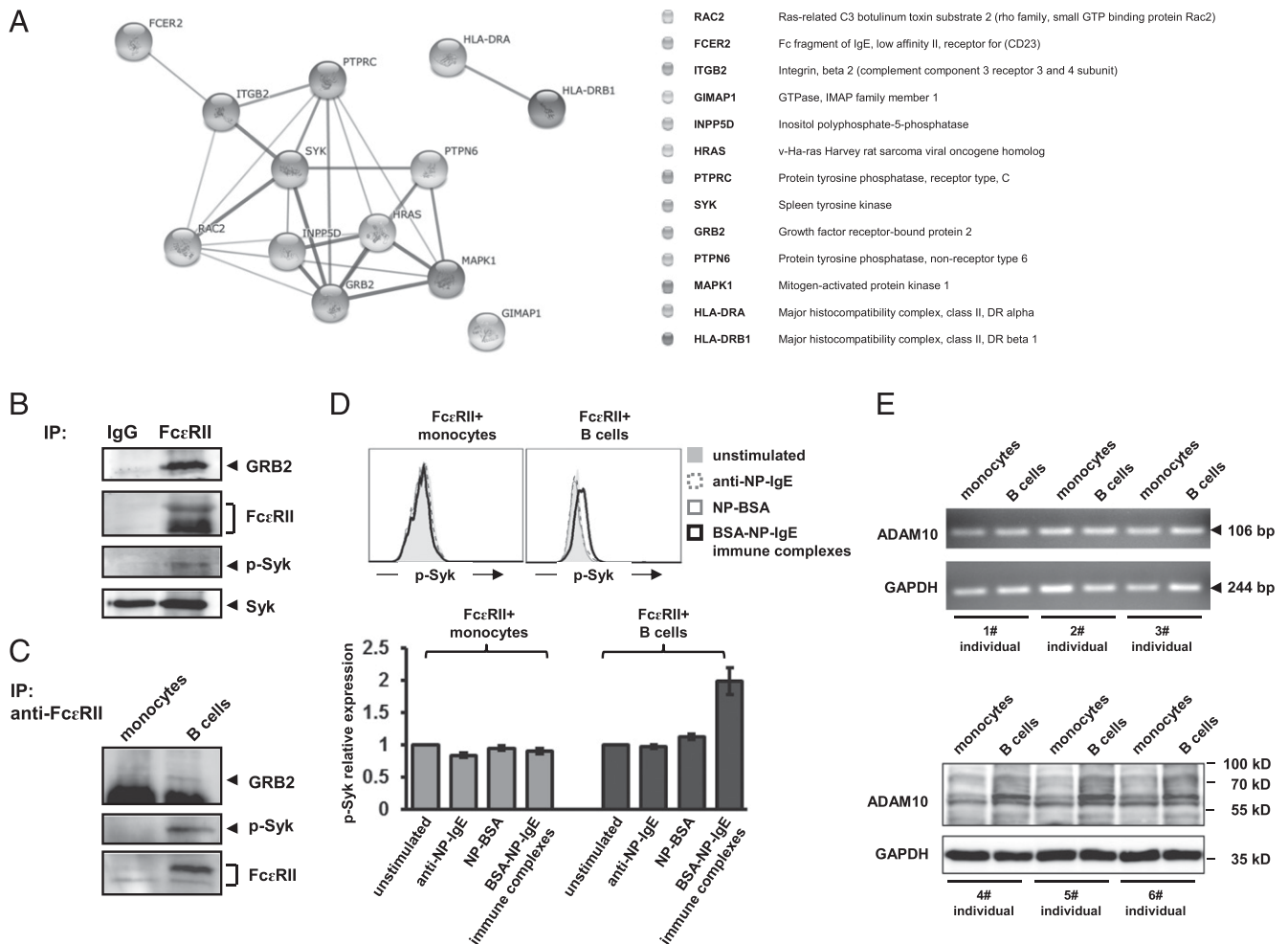


**FIGURE 6.** Identification of Syk as an important adaptor of FcεRII-mediated signals in human B cells. PBMCs were cultured with IL-4 for 2 d to boost the expression of FcεRII. **(A)** After BSA-NP-IgE immune complex stimulation, components of activated FcεRII complexes were immunoprecipitated with anti-FcεRII Ab and collected for MS analysis. A network of molecules found to be associated with B cell activation and Ag presentation is shown. **(B)** Molecules immunoprecipitated with activated FcεRII of human PBMCs after stimulation of BSA-NP-IgE immune complexes. **(C)** Isolated monocytes and B cells were stimulated with IL-4 for 2 d, and cells were stimulated with BSA-NP-IgE immune complexes and subjected to immunoprecipitation and immunoblotting analysis. Molecules immunoprecipitated with activated FcεRII of monocytes and B cells are shown. **(D)** After 2 d of culture with IL-4, human PBMCs were rested in medium containing 2% FCS at room temperature for 1 h. Then the cells were stimulated with anti-NP-IgE, NP-BSA, or BSA-NP-IgE immune complexes for 10 min and subjected to flow cytometric analysis. Representative intracellular phospho-Syk levels (upper panel) and relative expression of phospho-Syk calculated by median fluorescence intensity (lower panel) of CD14<sup>+</sup>FcεRII<sup>+</sup> monocytes and CD19<sup>+</sup>FcεRII<sup>+</sup> B cells from three independent experiments. **(E)** mRNA (upper panel) and protein (lower panel) expression of ADAM10 in human monocytes and B cells after IL-4 incubation. Results in (B) and (C) are representative of independent experiments from  $n = 3$  donors.

of the local milieu (60). Monocytes from healthy donors do not express high levels of FcεRII, whereas FcεRII expression can be induced by inflammatory milieu. Indeed, monocytes from atopic patients upregulate the expression of FcεRII (25). In our study, after stimulation with IL-4, human monocytes expressed high levels of surface FcεRIIb (Fig. 2); however, the elevated expression of FcεRIIb did not result in increased FcεRII-mediated allergen uptake, as observed in B cells (Fig. 3). The data are in line with the observation that FcεRIIa, but not FcεRIIb, acts as a bidirectional transporter of IgE or Ag-IgE complexes (7). Furthermore, the experiments were performed at 37°C. At this temperature, FcεRII decreases IgE-binding capacity as a result of the dissociation of FcεRII trimers (13, 14). Hence, the decreased FcεRII-mediated Ag uptake may be contested with other Ag-uptake mechanisms and, thereby, the effect is immersed into the high Ag-uptake background of monocytes (Fig. 3). Although stimulation of FcεRII in human monocytes did not induce marked tyrosine phosphorylation of intracellular proteins in our study, our

data did not refute the function of FcεRII on human monocytes. It was shown that FcεRII-mediated signals in monocytic cells, such as monocytes, macrophages, and the human monocytic U937 cell line, promote inflammation by producing free radicals, NOs, and proinflammatory cytokines (24). Moreover, our results demonstrate that human monocytes highly express ADAM10, a major sheddase of FcεRII; this suggests that human monocytes, together with B cells, contribute to allergic inflammation through regulation of IgE by the release of sFcεRII (17–19).

The association of FcεRII with MHC-II molecules is well documented (61, 62). It was shown in a mouse model that administration of Ag, together with Ag-specific IgE, enhances T cell- and B cell-specific responses in an FcεRII-dependent manner (63). In addition, murine peripheral B cells are capable of mediating the transport of IgE-immune complexes to splenic follicles via FcεRII (64). In line with these studies, we showed that peripheral human B cells are capable of binding and taking up Bet v 1-IgE immune complexes via FcεRII.

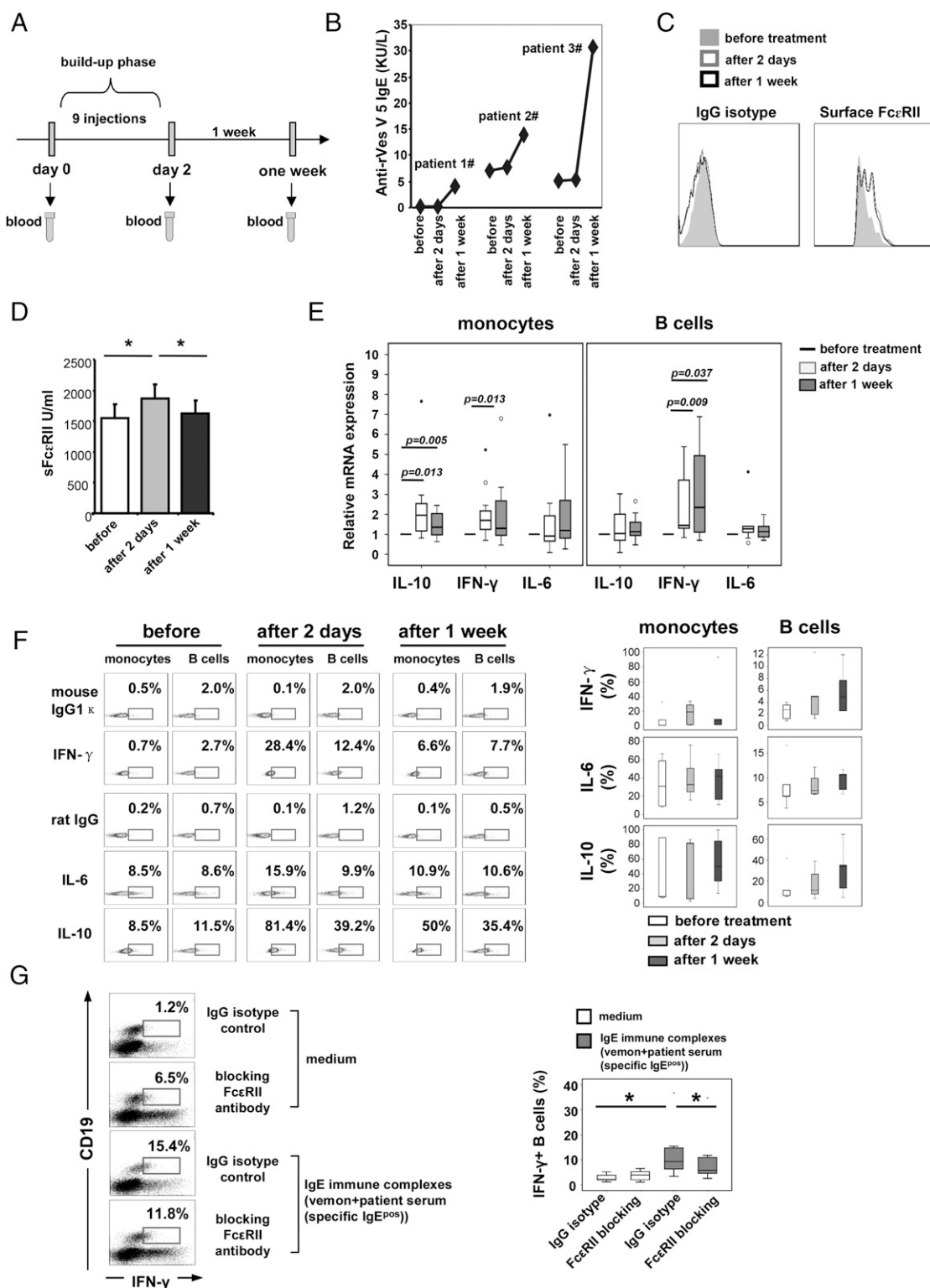


**FIGURE 7.** FcεRII-mediated signaling increases IFN- $\gamma$  production in B cells from allergic patients. **(A)** Blood samples from venom-allergic patients who underwent immunotherapy were collected as depicted. **(B)** Increased levels of serum-specific IgE against Ves v 5. Surface expression of FcεRII on PBMCs **(C)** and serum levels of sFcεRII of patients before, as well as 2 d and 1 wk after, the start of allergen injections **(D)** ( $n = 6$  donors). **(E)** Relative cytokine mRNA expression of monocytes and B cells from venom-allergic patients who underwent immunotherapy. Monocytes and B cells were isolated at the time points indicated and were subjected to TaqMan probe-based real-time PCR. 18S rRNA was used as internal control. Fold changes in mRNA expression over the expression of target gene before immunotherapy are shown (Wilcoxon test,  $n = 11$  donors). **(F)** Intracellular cytokine staining of freshly isolated monocytes and B cells from patients. One representative result of IFN- $\gamma$ , IL-6, and IL-10 production by CD14 $^{+}$  monocytes and CD19 $^{+}$  B cells is shown (left panels). Percentages of IFN- $\gamma$  $^{+}$ , IL-6 $^{+}$ , and IL-10 $^{+}$  cells of CD14 $^{+}$  monocytes and CD19 $^{+}$  B cells (right panels). Results are from  $n = 5$  independent experiments and are depicted as median  $\pm$  interquartile range. **(G)** Blockage of FcεRII decreased the percentage of IFN- $\gamma$ -producing B cells. PBMCs from patients with hymenoptera venom allergy were isolated and stimulated with venom-IgE immune complexes, with or without FcεRII blockage. After 48 h of culture, the cells were stimulated with 0.6% PHA for another 48 h before flow cytometric analysis. One representative result of the percentage of IFN- $\gamma$  $^{+}$  CD19 $^{+}$  B cells is shown (left panels). Statistical analysis of the percentage of IFN- $\gamma$  $^{+}$  B cells of total CD19 $^{+}$  B cells (right panel).  $n = 7$ , \* $p < 0.05$ , Wilcoxon test.

B cells are the major player in humoral immunity by producing Ag-specific Abs and innate immunity by their Ab-independent functions, including cytokine secretion. In our study, B cells from patients during the build-up phase of venom-specific immunotherapy expressed increased levels of IFN- $\gamma$ . Our results imply that activation of IFN- $\gamma$ -producing B cells by allergen-IgE immune complexes during the build-up phase of allergen-specific immunotherapy is a prerequisite for the successful treatment of allergic patients. Although IFN- $\gamma$  is primarily produced by CD4<sup>+</sup> T cells and NK cells, IFN- $\gamma$ -producing B cells were reported in infectious (46, 47, 49), lupus (50), and autoimmune arthritis (48) mouse models. IFN- $\gamma$  in B cells induces T-bet expression and, thereby, regulates the determination of parasite-specific B cells during *Plasmodium* infection (49). In a murine lupus model, intrinsic IFN- $\gamma$  produced by B cells contributes to the formation of spontaneous germinal centers, which are required for the spontaneous humoral autoimmunity (50). Depletion of intrinsic IFN- $\gamma$  signaling abolishes spontaneous autoimmune germinal centers and class-switched autoantibody production (51). Interestingly, the IFN- $\gamma$ -producing cells identified in the study (47) were CD11a<sup>hi</sup>Fc $\gamma$ RIII<sup>hi</sup> B cells, which also express Fc $\epsilon$ RII. Therefore, it is possible that IFN- $\gamma$ -producing B cells sense and react to IgE-allergen complexes via Fc $\epsilon$ RII. Because IFN- $\gamma$  enhances B cell activation (65, 66), it is likely that those IFN- $\gamma$ -producing B cells in human sense IgE-allergen immune complexes and secrete IFN- $\gamma$  to activate themselves in an autocrine manner or to activate other immune cells in a paracrine manner. However, the functions of those IFN- $\gamma$ -producing B cells in human allergic immune responses need to be investigated further.

Together, our study elucidates cell type-dependent functions of human monocytes and B cells in response to IgE immune complex stimulation via Fc $\epsilon$ RII. A better understanding of Fc $\epsilon$ RII-mediated responses of immune cells will be important for successful clinical control of allergic diseases.

## Disclosures

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## References

- Finkelman, F. D., and D. Vercelli. 2007. Advances in asthma, allergy mechanisms, and genetics in 2006. *J. Allergy Clin. Immunol.* 120: 544–550.
- Gould, H. J., and B. J. Sutton. 2008. IgE in allergy and asthma today. *Nat. Rev. Immunol.* 8: 205–217.
- Novak, N., S. Kraft, and T. Bieber. 2001. IgE receptors. *Curr. Opin. Immunol.* 13: 721–726.
- Acharya, M., G. Borland, A. L. Edkins, L. M. Maclellan, J. Matheson, B. W. Ozanne, and W. Cushley. 2010. CD23/Fc $\epsilon$ RII: molecular multi-tasking. *Clin. Exp. Immunol.* 162: 12–23.
- Kinet, J. P. 1999. The high-affinity IgE receptor (Fc $\epsilon$ RI): from physiology to pathology. *Annu. Rev. Immunol.* 17: 931–972.
- Novak, N. 2012. An update on the role of human dendritic cells in patients with atopic dermatitis. *J. Allergy Clin. Immunol.* 129: 879–886.
- Li, H., A. Nowak-Węgrzyn, Z. Charlop-Powers, W. Shreffler, M. Chehade, S. Thomas, G. Roda, S. Dahan, K. Sperber, and M. C. Berin. 2006. Transcytosis of IgE-antigen complexes by CD23a in human intestinal epithelial cells and its role in food allergy. *Gastroenterology* 131: 47–58.
- Palaniyandi, S., E. Tomei, Z. Li, D. H. Conrad, and X. Zhu. 2011. CD23-dependent transcytosis of IgE and immune complex across the polarized human respiratory epithelial cells. *J. Immunol.* 186: 3484–3496.
- van der Heijden, F. L., R. J. Joost van Neerven, M. van Katwijk, J. D. Bos, and M. L. Kapsenberg. 1993. Serum-IgE-facilitated allergen presentation in atopic disease. *J. Immunol.* 150: 3643–3650.
- van Neerven, R. J., T. Wikborg, G. Lund, B. Jacobsen, A. Brinch-Nielsen, J. Arved, and H. Ipsen. 1999. Blocking antibodies induced by specific allergy vaccination prevent the activation of CD4<sup>+</sup> T cells by inhibiting serum-IgE-facilitated allergen presentation. *J. Immunol.* 163: 2944–2952.
- van Neerven, R. J., E. F. Knol, A. Ejmaes, and P. A. Würtzen. 2006. IgE-mediated allergen presentation and blocking antibodies: regulation of T-cell activation in allergy. *Int. Arch. Allergy Immunol.* 141: 119–129.
- Beavil, R. L., P. Graber, N. Aubonney, J. Y. Bonnefoy, and H. J. Gould. 1995. CD23/Fc $\epsilon$ RII and its soluble fragments can form oligomers on the cell surface and in solution. *Immunology* 84: 202–206.
- Chen, B. H., M. A. Kilmon, C. Ma, T. H. Caven, Y. Chan-Li, A. E. Shelburne, R. M. Tombes, E. Roush, and D. H. Conrad. 2003. Temperature effect on IgE binding to CD23 versus Fc $\epsilon$ RII. *J. Immunol.* 170: 1839–1845.
- Kilmon, M. A., A. E. Shelburne, Y. Chan-Li, K. L. Holmes, and D. H. Conrad. 2004. CD23 trimers are preassociated on the cell surface even in the absence of its ligand, IgE. *J. Immunol.* 172: 1065–1073.
- Lemieux, G. A., F. Blumenkron, N. Yeung, P. Zhou, J. Williams, A. C. Grammer, R. Petrovich, P. E. Lipsky, M. L. Moss, and Z. Werb. 2007. The low affinity IgE receptor (CD23) is cleaved by the metalloproteinase ADAM10. *J. Biol. Chem.* 282: 14836–14844.
- Weskamp, G., J. W. Ford, J. Sturgill, S. Martin, A. J. Docherty, S. Swendeman, N. Broadway, D. Hartmann, P. Saftig, S. Umland, et al. 2006. ADAM10 is a principal ‘shedase’ of the low-affinity immunoglobulin E receptor CD23. *Nat. Immunol.* 7: 1293–1298.
- Aubry, J. P., S. Pochon, P. Graber, K. U. Jansen, and J. Y. Bonnefoy. 1992. CD21 is a ligand for CD23 and regulates IgE production. *Nature* 358: 505–507.
- Bonnefoy, J. Y., J. F. Gauchat, P. Life, P. Graber, J. P. Aubry, and S. Lecoanet-Henchoz. 1995. Regulation of IgE synthesis by CD23/CD21 interaction. *Int. Arch. Allergy Immunol.* 107: 40–42.
- Pongratz, G., J. W. McAlees, D. H. Conrad, R. S. Erbe, K. M. Haas, and V. M. Sanders. 2006. The level of IgE produced by a B cell is regulated by norepinephrine in a p38 MAPK- and CD23-dependent manner. *J. Immunol.* 177: 2926–2938.
- Kolb, J. P., A. Abadie, N. Paul-Eugene, M. Capron, M. Sarfati, B. Dugas, and G. Delespesse. 1993. Ligation of CD23 triggers cyclic AMP generation in human B lymphocytes. *J. Immunol.* 150: 4798–4809.
- Kolb, J. P., D. Renard, B. Dugas, E. Genot, E. Petit-Koskas, M. Sarfati, G. Delespesse, and J. Poggioli. 1990. Monoclonal anti-CD23 antibodies induce a rise in [Ca<sup>2+</sup>]<sub>i</sub> and polyphosphoinositide hydrolysis in human activated B cells. Involvement of a Gp protein. *J. Immunol.* 145: 429–437.
- Fournier, S., G. Delespesse, M. Rubio, G. Biron, and M. Sarfati. 1992. CD23 antigen regulation and signaling in chronic lymphocytic leukemia. *J. Clin. Invest.* 89: 1312–1321.
- Paul-Eugene, N., J. P. Kolb, A. Abadie, J. Gordon, G. Delespesse, M. Sarfati, J. M. Mencia-Huerta, P. Braquet, and B. Dugas. 1992. Ligation of CD23 triggers cAMP generation and release of inflammatory mediators in human monocytes. *J. Immunol.* 149: 3066–3071.
- Ten, R. M., M. J. McKinstry, G. D. Bren, and C. V. Paya. 1999. Signal transduction pathways triggered by the Fc $\epsilon$ RIIb receptor (CD23) in human monocytes lead to nuclear factor- $\kappa$ B activation. *J. Allergy Clin. Immunol.* 104: 376–387.
- Williams, J., S. Johnson, J. J. Mascali, H. Smith, L. J. Rosenwasser, and L. Borish. 1992. Regulation of low affinity IgE receptor (CD23) expression on mononuclear phagocytes in normal and asthmatic subjects. *J. Immunol.* 149: 2823–2829.
- Gagro, A., S. Rabatić, A. Tresćec, D. Dekaris, and M. Medar-Lasić. 1993. Expression of lymphocytes Fc $\epsilon$ RII/CD23 in allergic children undergoing hyposensitization. *Int. Arch. Allergy Immunol.* 101: 203–208.
- Rabatić, S., A. Gagro, and M. Medar-Lasić. 1993. CD21-CD23 ligand pair expression in children with allergic asthma. *Clin. Exp. Immunol.* 94: 337–340.
- Rosenwasser, L. J., W. W. Busse, R. G. Lizambri, T. A. Olejnik, and M. C. Totoritis. 2003. Allergic asthma and an anti-CD23 mAb (IDEC-152): results of a phase I, single-dose, dose-escalating clinical trial. *J. Allergy Clin. Immunol.* 112: 563–570.
- Plater-Zyberk, C., and J. Y. Bonnefoy. 1995. Marked amelioration of established collagen-induced arthritis by treatment with antibodies to CD23 in vivo. *Nat. Med.* 1: 781–785.
- Yang, P. C., M. C. Berin, L. C. Yu, D. H. Conrad, and M. H. Perdue. 2000. Enhanced intestinal trans epithelial antigen transport in allergic rats is mediated by IgE and CD23 (Fc $\epsilon$ RII). *J. Clin. Invest.* 106: 879–886.
- Akdis, M., and C. A. Akdis. 2014. Mechanisms of allergen-specific immunotherapy: multiple suppressor factors at work in immune tolerance to allergens. *J. Allergy Clin. Immunol.* 133: 621–631.
- Golden, D. B., et al. 2011. Stinging insect hypersensitivity: a practice parameter update 2011. *J. Allergy Clin. Immunol.* 127: 852–854.e1–23.
- Krishna, M. T., P. W. Ewan, L. Diwakar, S. R. Durham, A. J. Frew, S. C. Leech, and S. M. Nasser. British Society for Allergy and Clinical Immunology. 2011. Diagnosis and management of hymenoptera venom allergy: British Society for Allergy and Clinical Immunology (BSACI) guidelines. *Clin. Exp. Allergy* 41: 1201–1220.
- Bonifazi, F., M. Jutel, B. M. Biló, J. Birnbaum, and U. Muller, EAACI Interest Group on Insect Venom Hypersensitivity. 2005. Prevention and treatment of hymenoptera venom allergy: guidelines for clinical practice. *Allergy* 60: 1459–1470.
- Laffer, S., E. Hogbom, K. H. Roux, W. R. Sperr, P. Valent, H. C. Bankl, L. Vangelista, F. Kricek, D. Kraft, H. Grönlund, and R. Valenta. 2001. A molecular model of type I allergy: identification and characterization of a non-anaphylactic anti-human IgE antibody fragment that blocks the IgE-Fc $\epsilon$ RI interaction and reacts with receptor-bound IgE. *J. Allergy Clin. Immunol.* 108: 409–416.
- Kwiek, B., W. M. Peng, J. P. Allam, A. Langner, T. Bieber, and N. Novak. 2008. Tacrolimus and TGF- $\beta$  act synergistically on the generation of langerhans cells. *J. Allergy Clin. Immunol.* 122: 126–132, 132.e1.
- Schmittgen, T. D., and K. J. Livak. 2008. Analyzing real-time PCR data by the comparative C(T) method. *Nat. Protoc.* 3: 1101–1108.

38. McCulloch, D. R., P. Akl, H. Samaratunga, A. C. Herington, and D. M. Odorico. 2004. Expression of the disintegrin metalloprotease, ADAM-10, in prostate cancer and its regulation by dihydrotestosterone, insulin-like growth factor I, and epidermal growth factor in the prostate cancer cell model LNCaP. *Clin. Cancer Res.* 10: 314–323.
39. Huang, W., B. T. Sherman, and R. A. Lempicki. 2009. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nat. Protoc.* 4: 44–57.
40. Novak, N., C. Tepel, S. Koch, K. Brix, T. Bieber, and S. Kraft. 2003. Evidence for a differential expression of the FcεpsilonRIγ chain in dendritic cells of atopic and nonatopic donors. *J. Clin. Invest.* 111: 1047–1056.
41. Karagiannis, S. N., J. K. Warrack, K. H. Jennings, P. R. Murdock, G. Christie, K. Moulder, B. J. Sutton, and H. J. Gould. 2001. Endocytosis and recycling of the complex between CD23 and HLA-DR in human B cells. *Immunology* 103: 319–331.
42. Hunter, T. 2009. Tyrosine phosphorylation: thirty years and counting. *Curr. Opin. Cell Biol.* 21: 140–146.
43. Cheng, A. M., and A. C. Chan. 1997. Protein tyrosine kinases in thymocyte development. *Curr. Opin. Immunol.* 9: 528–533.
44. Kurosaki, T. 1997. Molecular mechanisms in B cell antigen receptor signaling. *Curr. Opin. Immunol.* 9: 309–318.
45. Bussmann, C., J. Xia, J. P. Allam, L. Maintz, T. Bieber, and N. Novak. 2010. Early markers for protective mechanisms during rush venom immunotherapy. *Allergy* 65: 1558–1565.
46. Ganapamo, F., V. A. Dennis, and M. T. Philipp. 2001. CD19(+) cells produce IFN-γ in mice infected with *Borrelia burgdorferi*. *Eur. J. Immunol.* 31: 3460–3468.
47. Bao, Y., X. Liu, C. Han, S. Xu, B. Xie, Q. Zhang, Y. Gu, J. Hou, L. Qian, C. Qian, et al. 2014. Identification of IFN-γ-producing innate B cells. *Cell Res.* 24: 161–176.
48. Olalekan, S. A., Y. Cao, K. M. Hamel, and A. Finnegan. 2015. B cells expressing IFN-γ suppress Treg-cell differentiation and promote autoimmune experimental arthritis. *Eur. J. Immunol.* 45: 988–998.
49. Guthmiller, J. J., A. C. Graham, R. A. Zander, R. L. Pope, and N. S. Butler. 2017. Cutting edge: IL-10 is essential for the generation of germinal center B cell responses and anti-plasmodium humoral immunity. *J. Immunol.* 198: 617–622.
50. Domeier, P. P., S. B. Chodiseti, C. Soni, S. L. Schell, M. J. Elias, E. B. Wong, T. K. Cooper, D. Kitamura, and Z. S. Rahman. 2016. IFN-γ receptor and STAT1 signaling in B cells are central to spontaneous germinal center formation and autoimmunity. *J. Exp. Med.* 213: 715–732.
51. Jackson, S. W., H. M. Jacobs, T. Arkatkar, E. M. Dam, N. E. Scharping, N. S. Kolhatkar, B. Hou, J. H. Buckner, and D. J. Rawlings. 2016. B cell IFN-γ receptor signaling promotes autoimmune germinal centers via cell-intrinsic induction of BCL-6. *J. Exp. Med.* 213: 733–750.
52. Chomarat, P., J. Briolay, J. Banchereau, and P. Miossec. 1993. Increased production of soluble CD23 in rheumatoid arthritis, and its regulation by interleukin-4. *Arthritis Rheum.* 36: 234–242.
53. Bansal, A. S., W. Ollier, M. N. Marsh, R. S. Pumphrey, and P. B. Wilson. 1993. Variations in serum sCD23 in conditions with either enhanced humoral or cell-mediated immunity. *Immunology* 79: 285–289.
54. Awan, F. T., P. Hillmen, A. Hellmann, T. Robak, S. G. Hughes, D. Trone, M. Shannon, I. W. Flinn, and J. C. Byrd, LUCID trial investigators. 2014. A randomized, open-label, multicentre, phase 2/3 study to evaluate the safety and efficacy of lumiliximab in combination with fludarabine, cyclophosphamide and rituximab versus fludarabine, cyclophosphamide and rituximab alone in subjects with relapsed chronic lymphocytic leukaemia. *Br. J. Haematol.* 167: 466–477.
55. Byrd, J. C., T. J. Kipps, I. W. Flinn, J. Castro, T. S. Lin, W. Wierda, N. Heerema, J. Woodworth, S. Hughes, S. Tangri, et al. 2010. Phase 1/2 study of lumiliximab combined with fludarabine, cyclophosphamide, and rituximab in patients with relapsed or refractory chronic lymphocytic leukemia. *Blood* 115: 489–495.
56. Chan, M. A., N. M. Gigliotti, P. Matangkasombut, S. B. Gauld, J. C. Cambier, and L. J. Rosenwasser. 2010. CD23-mediated cell signaling in human B cells differs from signaling in cells of the monocytic lineage. *Clin. Immunol.* 137: 330–336.
57. Armand, M., M. Rubio, G. Delespesse, and M. Sarfati. 1995. Soluble CD23 directly activates monocytes to contribute to the antigen-independent stimulation of resting T cells. *J. Immunol.* 155: 4868–4875.
58. Cheng, A. M., B. Rowley, W. Pao, A. Hayday, J. B. Bolen, and T. Pawson. 1995. Syk tyrosine kinase required for mouse viability and B-cell development. *Nature* 378: 303–306.
59. Cornall, R. J., A. M. Cheng, T. Pawson, and C. C. Goodnow. 2000. Role of Syk in B-cell development and antigen-receptor signaling. *Proc. Natl. Acad. Sci. USA* 97: 1713–1718.
60. Geissmann, F., M. G. Manz, S. Jung, M. H. Sieweke, M. Merad, and K. Ley. 2010. Development of monocytes, macrophages, and dendritic cells. *Science* 327: 656–661.
61. Flores-Romo, L., G. D. Johnson, A. A. Ghaderi, D. R. Stanworth, A. Veronesi, and J. Gordon. 1990. Functional implication for the topographical relationship between MHC class II and the low-affinity IgE receptor: occupancy of CD23 prevents B lymphocytes from stimulating allogeneic mixed lymphocyte responses. *Eur. J. Immunol.* 20: 2465–2469.
62. Bonnefoy, J. Y., O. Guillot, H. Spits, D. Blanchard, K. Ishizaka, and J. Banchereau. 1988. The low-affinity receptor for IgE (CD23) on B lymphocytes is spatially associated with HLA-DR antigens. *J. Exp. Med.* 167: 57–72.
63. Getahun, A., F. Hjelm, and B. Heyman. 2005. IgE enhances antibody and T cell responses in vivo via CD23+ B cells. *J. Immunol.* 175: 1473–1482.
64. Hjelm, F., M. C. Karlsson, and B. Heyman. 2008. A novel B cell-mediated transport of IgE-immune complexes to the follicle of the spleen. *J. Immunol.* 180: 6604–6610.
65. Morikawa, K., H. Kubagawa, T. Suzuki, and M. D. Cooper. 1987. Recombinant interferon-α, -β, and -γ enhance the proliferative response of human B cells. *J. Immunol.* 139: 761–766.
66. Shen, P., and S. Fillatreau. 2015. Antibody-independent functions of B cells: a focus on cytokines. *Nat. Rev. Immunol.* 15: 441–451.