

Human Basophils Express the Glycosylphosphatidylinositol-Anchored Low-Affinity IgG Receptor FcγRIIB (CD16B)¹

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Basophils express not only high-affinity IgE receptors, but also low-affinity IgG receptors. Which, among these receptors, are expressed by human basophils is poorly known. Low-affinity IgG receptors comprise CD32 (FcγRIIA, FcγRIIB, and FcγRIIC) and CD16 (FcγRIIA and FcγRIIB). FcγRIIA, FcγRIIC, and FcγRIIA are activating receptors, FcγRIIB are inhibitory receptors, FcγRIIB are GPI-anchored receptors whose function is poorly understood. Basophils were reported to express FcγRII, but not FcγRIII. We aimed at further identifying basophil IgG receptors. Basophils from normal donors and from patients suffering from an allergic skin disease (atopic dermatitis), allergic respiratory diseases (allergic rhinitis and asthma), or a non-allergic skin disease (chronic urticaria) were examined. We found that normal basophils contain FcγRIII transcripts and express FcγRIIB, but not FcγRIIA, which were detected on 24–81% basophils from normal donors and on 12–100% basophils from patients. Noticeably, the proportion of FcγRIIB⁺ basophils was significantly lower in atopic dermatitis patients than in other subjects. This decreased FcγRIII expression was not correlated with an activated phenotype of basophils in atopic dermatitis patients, although FcγRIIB expression was down-regulated upon basophil activation by anti-IgE. Our results challenge the two dogmas 1) that basophils do not express FcγRIII and 2) that FcγRIIB is exclusively expressed by neutrophils. They suggest that a proportion of basophils may be lost during enrichment procedures in which FcγRIII⁺ cells are discarded by negative sorting using anti-CD16 Abs. They unravel an unexpected complexity of IgG receptors susceptible to modulate basophil activation. They identify a novel systemic alteration in atopic dermatitis. *The Journal of Immunology*, 2009, 182: 2542–2550.

Basophils are increasingly recognized as critical cells in allergy. Like mast cells, they contain and, upon stimulation, they release preformed granular vasoactive amines, they synthesize lipid-derived mediators, and they secrete high amounts of Th2 cytokines (1). As a consequence, basophils are thought to play pivotal roles not only as the effectors of acute reactions such as anaphylaxis but also, by promoting Th2 polarization, as the inducers of an atopic phenotype (2). Additionally, basophils were recently shown to promote IgE-dependent chronic allergic inflammation in mice (3).

Basophils can be activated by numerous extracellular stimuli which engage cell-activating receptors. Among these are receptors for the Fc portion of Abs (FcRs). Activating FcRs include IgE and IgG receptors. Basophils express high-affinity IgE receptors (FcεRI), which are well known as the triggers of IgE-dependent

allergic reactions and anaphylaxis (4). The activating properties of FcεRI depend on the presence of ITAMs in the intracytoplasmic domains of Fcγ and Fcβ, the two FcR common subunits with which they constitutively associate (5). Mast cells and basophils also express low-affinity IgG receptors. Mouse, but not human mast cells express FcγRIIA, which also associate with Fcγ and Fcβ in these cells. FcγRIIA activate mouse mast cells, both in vitro (6) and in vivo (7). Whether mouse basophils express FcγRIIA is not known. Basophils were however recently reported to account for IgG-induced, but not IgE-induced passive systemic anaphylaxis in mice, whereas mast cells accounted for IgE-induced, but not IgG-induced anaphylaxis (8). Mouse basophils therefore express functional activating IgG receptors. Whether a similar situation applies to humans is not known. Human, but not mouse, mast cells and basophils express FcγRIIA/C (9), which do not associate with Fcγ or Fcβ, but which contain an ITAM in their own intracytoplasmic domain. FcγRIIA were shown to activate mast cells in RBL-2H3 transfectants (9) and in human skin-derived mast cells (10). Whether they can activate human basophils is not known. Finally, although not formally demonstrated, human basophils probably also express inhibitory IgG receptors (FcγRIIB). The coengagement of IgG receptors with IgE receptors on basophils by allergen-IgG Ab complexes was indeed found to inhibit IgE-induced histamine release (9, 11), as it had been previously shown in mouse mast cells (12). FcγRs are therefore potential positive and/or negative regulators of basophil activation.

The CD nomenclature is often used to designate human FcRs. Using this nomenclature, basophils were described to express CD32, but not CD16 (13–15). CD32 refers to FcγRII whereas CD16 refers to FcγRIII. CD32 therefore includes FcγRIIA, FcγRIIB, and FcγRIIC, i.e., one inhibitory and two activating receptors. One anti-CD32 mAb specifically recognizes FcγRIIA, another recognizes FcγRIIB and FcγRIIC, and others, such as

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Table I. Patients included in this study

Patient	Age (years)	Sex	Disease	Allergens	Severity	Total IgE (kU/L)
1	21	F ^a	Atopic dermatitis	Polysensitized	Moderate	402
2	26	F	Atopic dermatitis	Polysensitized	Moderate	4620
3	68	M	Atopic dermatitis	Polysensitized	Moderate	16556
4	30	F	Atopic dermatitis	Polysensitized	Moderate	2454
5	13	F	Atopic dermatitis	Polysensitized	Severe	4273
6	31	M	Chronic urticaria	None	Moderate	57
7	57	M	Chronic urticaria ^b	None	Moderate	105
8	26	F	Chronic urticaria	None	Moderate	126
9	26	F	Chronic urticaria	None	Dermographia	266
10	48	M	Chronic urticaria	None	Moderate	128
11	63	F	Chronic urticaria ^b	None	Moderate	158
12	65	F	Chronic urticaria ^b	None	Moderate	3
13	31	M	Rhinitis	Pollen (grass, birch)	Moderate	128
14	53	M	Rhinitis	Pollen (grass, birch)	Moderate	200
15	41	F	Rhinitis	Mites, cat	Moderate	146
16	36	F	Rhinitis	Pollen (grass, birch), mites	Moderate	140
17	25	M	Rhinitis	Mites, cat	Moderate	137
18	28	M	Rhinitis	Pollen (grass)	Moderate	609
19	21	F	Rhinitis	Pollen (grass, birch)	Moderate	183
20	19	F	Asthma	Pollen (grass)	Persistent/moderate	628
21	68	F	Asthma	Mites	Persistent/moderate	110
22	46	F	Asthma	Pollen (grass, birch), cat	Persistent/moderate	684
23	45	M	Asthma	Mites	Persistent/moderate	98
24	58	F	Asthma	Pollen (grass), mites	Persistent/moderate	117
25	47	M	Asthma	Mites	Persistent/moderate	60

^a F, Female; M, male.^b Associated with autoimmune thyroiditis.

mAb FLI8.26 (16), recognize all three FcγRII subtypes. There is no commercially available FcγRIIB- or FcγRIIC-specific Ab. Likewise, CD16 includes two FcγRIII isoforms. FcγRIIIA (CD16A) is an FcRγ-associated (17) transmembrane receptor (18) expressed by monocytes (19), NK cells (20), and NKT cells (21). FcγRIIIB (CD16B) is a GPI-anchored receptor which does not associate with FcRγ and has no intracytoplasmic domain (22). FcγRIIIB is thought to be exclusively expressed by human polymorphonuclear neutrophils (23). Several anti-FcγRIII Abs, such as mAbs 3G8 (24) and LNK16 (25), recognize both FcγRIIIA and FcγRIIIB. No FcγRIIIA- or FcγRIIIB-specific Ab is available.

Studies on basophils have been hampered by the low number of these cells in peripheral blood and by the lack of reliable in vitro models of mature basophils. Various methods of basophil enrichment have therefore been developed using centrifugation (e.g., elutriation) and/or sorting procedures based on the differential expression of membrane molecules by basophils. These enrichment techniques are not without inconvenience. Basophils may be altered (e.g., activated) by positive selection procedures while some may be discarded by negative selection procedures. Basophils can be identified among other blood leukocytes by flow cytometry using CD203c. Differing from other membrane molecules, CD203c is indeed a specific marker of blood basophils (14, 26). It is constitutively expressed and up-regulated upon basophil activation (26). CD203c is therefore used as a positive criterion for identifying basophils and its increased expression as an indicator of basophil activation. Using CD203c as a marker, we undertook an investigation of FcγRs expressed by human basophils in blood samples from normal and allergic individuals.

We found that blood basophils express not only FcγRII, as expected, but also FcγRIII. We demonstrate that basophils detectably express FcγRIIIB, but not FcγRIIIA, that FcγRIIIB is detectably expressed by at least one-fourth of basophils in all normal donors, and that the percentage of FcγRIIIB⁺ basophils

is significantly lower in atopic dermatitis patients than in normal donors.

Materials and Methods

Subjects

Healthy controls. Blood from normal donors was obtained from the Centre Necker-Cabanel of the Etablissement Français du Sang, (Paris, France).

Patients. Peripheral blood was collected, within the frame of disease exploration and with informed consent, from 25 patients who visited the Allergy Outpatient Clinic of the Centre Médical de l'Institut Pasteur between May and September 2007. These included five patients with atopic dermatitis, seven patients with rhinitis, six patients with allergic asthma, and seven patients with chronic urticaria (Table I). Atopic dermatitis fulfilled the clinical criteria of Hanifin and Rajka (27) and Hanifin (28). Its severity was assessed by the Scoring Atopic Dermatitis Index (29). Rhinitis and allergic asthma were classified according to the guidelines of the Allergic Rhinitis and Its Impact on Asthma (30) and of the Global Initiative for Asthma (31). Chronic urticaria was defined as recurrent episodes of everyday hives for more than 6 mo with no identified trigger. Three among chronic urticaria patients had an associated autoimmune thyroiditis. All patients underwent skin prick tests with common aeroallergens. No patient received systemic immunosuppressive or corticosteroid treatment. The investigation was approved by the Biomedical Research Committee of the Institut Pasteur.

Serum IgE levels

Total serum IgE was measured by the enzyme immunoassay-based ACCESS kit (Beckman Coulter).

Abs and reagents

Labeled Abs. Abs and corresponding isotype controls used for immunofluorescence analysis are listed in Table II. PE-conjugated anti-CD3, anti-CD14, anti-CD19, anti-CD24, anti-CD56, MOPC21 and G155-178, and FITC-conjugated anti-CD16 (mAb 3G8 anti-FcγRIII), anti-CD32 (mAb FLI8.26 anti-FcγRII), MOPC21 and 27-35 were purchased from BD Biosciences. PE-conjugated anti-CD203c and 679.1Mc7 were from Beckman Coulter. Allophycocyanin-conjugated anti-CD16 (mAb 3G8 anti-FcγRIII) and control mouse monoclonal IgG1, biotinylated anti-CD16 (mAb LNK16 anti-FcγRIII) were from Serotec; allophycocyanin-conjugated

Table II. Abs used for immunofluorescence analysis

Label	Isotype	Ab (clone)	Isotype Control
FITC	Mouse IgG1	Anti-CD16 (3G8)	MOPC-21
	Mouse IgG2b	Anti-CD32 (FL18.26)	27-35
	Goat IgG	Anti-human IgE	Normal goat IgG
PE	Mouse IgG1	Anti-CD56 (B159)	MOPC-21
	Mouse IgG1	Anti-CD19 (HIB19)	MOPC-21
	Mouse IgG1	Anti-CD203c (97A6)	679.1Mc7
	Mouse IgG2a	Anti-CD14 (M5E2)	G155-178
	Mouse IgG2a	Anti-CD3 (HIT3a)	G155-178
	Mouse IgG2a	Anti-CD24 (ML5)	G155-178
Allophycocyanin	Mouse IgG1	Anti-CD16 (3G8)	MOPC-21
	Mouse IgG2b	Anti-Fc ϵ RI (AER-37)	27-35
Biotin	Mouse IgG1	Anti-CD16 (LNK16)	—

anti-Fc ϵ RI was from eBioscience. FITC-conjugated polyclonal goat anti-human IgE was from Sigma-Aldrich and FITC-conjugated normal polyclonal goat IgG was from Jackson ImmunoResearch Laboratories.

Nonlabeled Abs. Polyclonal rabbit anti-human IgE was from Dako-Cytomation. F(ab')₂ of rabbit anti-mouse IgG (RAM)⁴ were from Jackson ImmunoResearch Laboratories. 3G8 F(ab')₂ were prepared by pepsin digestion of 3G8 IgG Abs purified from hybridoma supernatant by affinity chromatography on protein G-Sepharose.

Reagents. UltraAvidin-FITC was from Leinco Technologies.

Immunofluorescence

Whole blood cells were depleted of RBCs by hypotonic lysis in buffer containing 77 mM NH₄Cl, 3.6 mM K₂CO₃, and 0.4 mM EDTA and were washed in cold PBS containing 0.5% BSA (PBS-BSA). Cells were incubated with PE-conjugated, FITC-conjugated, and/or allophycocyanin-conjugated Abs for 15 min at 0°C. Cell fluorescence was analyzed by flow cytometry in gates (see Fig. 2) using a FACSCalibur (BD Biosciences). For basophil analysis, a constant number of 1000 CD203c⁺ cells was acquired.

Cell sorting

PBMCs were isolated from whole human blood by Ficoll-Paque density centrifugation. Basophils were identified using allophycocyanin-conjugated anti-Fc ϵ RI and PE-conjugated anti-CD203c Abs and separated using a MoFlo sorter (DakoCytomation). To assess the purity of sorted cells, cytopspins were prepared and cells were colored with Giemsa stain (Fluka). Images were acquired using a Zeiss Axioplan microscope equipped with a Zeiss Axiovision HRC camera and Axiovision 4.4 software.

RT-PCR

RT-PCR was performed on cDNA prepared with RNA from sorted human basophils and, as positive and negative controls, on cDNA from blood leukocytes and from Chinese hamster ovary (CHO) cells or CHO transfectants expressing human Fc γ RIIA, Fc γ RIIB, Fc γ RIIA, or Fc γ RIIIB (32). cDNA was produced using Superscript II Reverse Transcriptase (Invitrogen) either with oligo(dT) or random hexamers. The following primers were used: Fc γ RIII forward, 5'-TCAATGTTTGTCTTCACAG-3'; Fc γ RIII reverse, 5'-ATTCACCTGAGGTGTACAG-3'; tubulin forward, 5'-TGTTCTGACCTGGAACCCAC-3'; and tubulin reverse, 5'-CTCAACCACAGCAGTGGAACC-3'.

Phosphatidylinositol-specific phospholipase C (PI-PLC) digestion

RBC-depleted blood cells were incubated with 0.5 IU/ml PI-PLC (Sigma-Aldrich) for 30 min at 37°C. The enzymatic reaction was stopped by washing cells in cold PBS-BSA. Cells were stained with fluorescent Abs and analyzed by flow cytometry. PI-PLC treatment had no effect on CD203c expression by basophils.

Basophil activation

Anti-IgE induced. Blood cells were incubated with 10 μ g/ml anti-IgE Abs for 30 min at 37°C before RBCs were depleted by hypotonic lysis. They

were then stained with anti-CD203c-PE and fluorescence was analyzed by flow cytometry.

Anti-CD16 induced. RBC-depleted cells were incubated with 10 μ g/ml 3G8 F(ab')₂ for 30 min at 37°C, washed, and incubated with 30 μ g/ml RAM F(ab')₂ for 30 min at 37°C. Stimulation was stopped by washing cells in cold PBS-BSA. Cells were stained with PE-conjugated anti-CD203c Abs and analyzed by flow cytometry.

Statistical analysis

Data were analyzed with the Statistical Analysis System package for Macintosh (SAS Institute). A nonparametric Mann-Whitney *U* test was used to compare normal donors and patients. Correlations were analyzed using the Pearson's correlation coefficient.

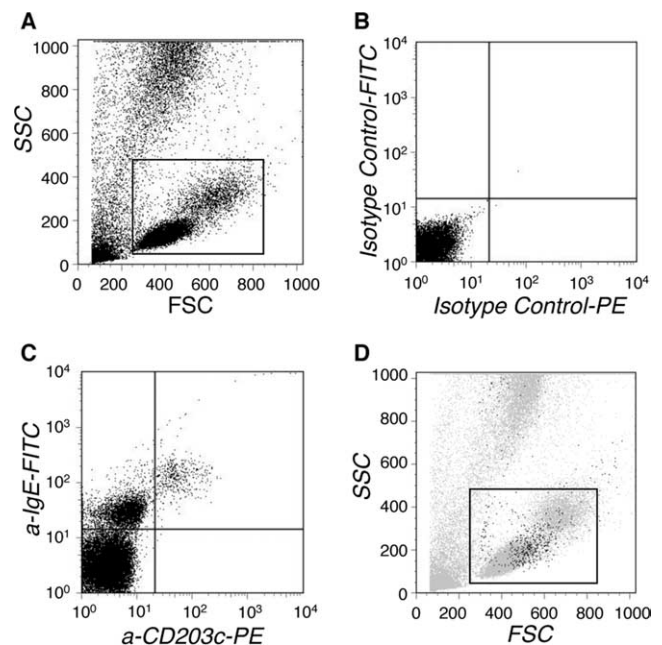


FIGURE 1. Identification of blood basophils by flow cytometry. **A**, FSC/SSC dot plot analysis of RBC-depleted blood cells from a normal donor. The box shows the gate used for basophil analysis. **B**, Aliquots of the same cells were incubated with FITC-conjugated normal goat IgG and PE-conjugated IgG1 679.1Mc7. FL1 and FL2 were analyzed in gated cells. **C**, Aliquots of the same cells were incubated with FITC-conjugated goat anti-human IgE and PE-conjugated anti-CD203c Abs. FL1 and FL2 were analyzed in the same gate. **D**, IgE⁺CD203c⁺ cells (black dots) were back-gated in the FSC/SSC dot plot of the whole cell population (gray dots). Doubly positive cells were concentrated within the gate used in **B** and **C**. This gate was used for basophil analysis in subsequent experiments.

⁴ Abbreviations used in this paper: RAM, rabbit anti-mouse Ig; MFI, mean fluorescence intensity; PI-PLC, phosphatidylinositol-specific phospholipase C; CHO, Chinese hamster ovary; SSC, side scatter; FSC, forward scatter.

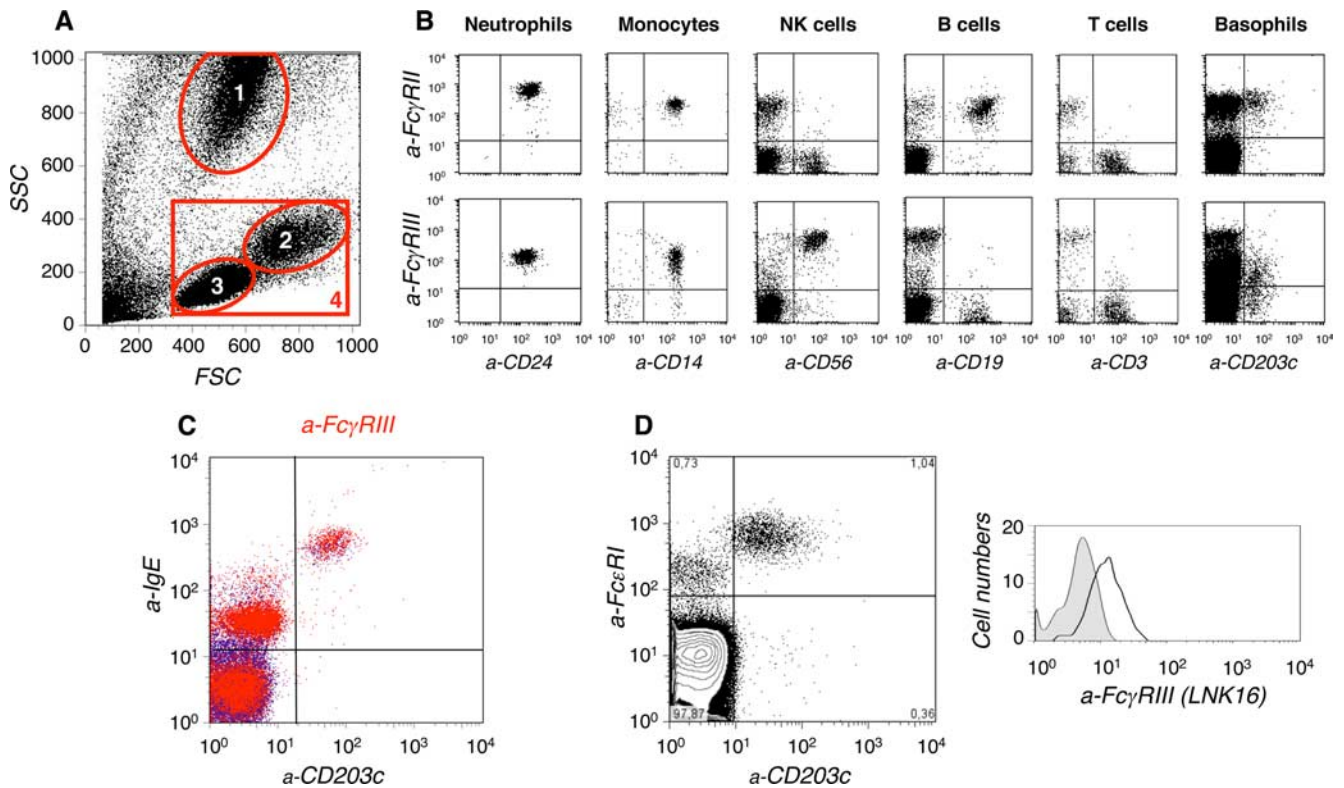


FIGURE 2. Basophils express FcγRII and FcγRIII. *A*, Representative FCS/SSC dot plot analysis of RBC-depleted blood cells from a normal donor showing the four gates used for analysis of cell populations shown in *B* and *C*. *B*, Cells were incubated with PE-conjugated anti-CD24, anti-CD14, anti-CD56, anti-CD19, anti-CD3, anti-CD203c Abs or isotype controls and with FITC-conjugated anti-FcγRII (mAb FL18.26), anti-FcγRIII (mAb 3G8) Abs, or isotype controls. Neutrophils were analyzed in gate 1, monocytes were analyzed in gate 2, NK cells, B cells, and T cells were analyzed in gate 3, and basophils were analyzed in gate 4. FL1 and FL2 recorded with isotype controls (data not shown) were used to set up horizontal and vertical bars, respectively, defining fluorescence thresholds separating negative from positive cells. *C*, Cells were incubated with PE-conjugated anti-CD203c, FITC-conjugated goat anti-human IgE, and allophycocyanin-conjugated anti-FcγRIII (mAb 3G8) Abs. Basophils were analyzed in gate 4. FL1 and FL2 recorded with isotype controls (data not shown) were used to set up horizontal and vertical bars, respectively, defining fluorescence thresholds separating negative from positive cells. FcγRIII⁺ cells are shown in red. *D*, PBMCs were purified from normal blood by Ficoll density centrifugation. Cells were incubated first with biotinylated anti-FcγRIII (mAb LNK16) Abs or without, then with PE-conjugated anti-CD203c Abs, allophycocyanin-conjugated anti-FcεRI Abs, and FITC-conjugated avidin. The contour plot shows the staining of cells with anti-FcεRI and anti-CD203c Abs. The histogram shows the staining of FcεRI⁺ CD203c⁺ cells with anti-FcγRIII Abs (open histogram) over the control without primary Ab (filled histogram).

Results

Human basophils express FcγRIII

CD203c is a specific marker of human basophils (14, 26). Blood basophils also express FcεRI, a proportion of which is occupied by IgE. When analyzed by flow cytometry in RBC-depleted blood cells previously incubated with PE-conjugated anti-CD203c and FITC-conjugated anti-IgE Abs, IgE⁺CD203c⁺ cells were found in a region having a low side scatter (SSC) and an intermediate forward scatter (FSC) (Fig. 1). As expected, IgE⁺ cells included CD203c⁺ and CD203c⁻ cells. Cells other than basophils indeed express IgE receptors. By contrast, all CD203c⁺ cells were IgE⁺. CD203c alone is therefore sufficient to identify basophils in normal blood. The percentage of CD203c⁺ cells was $0.67 \pm 0.42\%$ ($n = 17$) in normal donors.

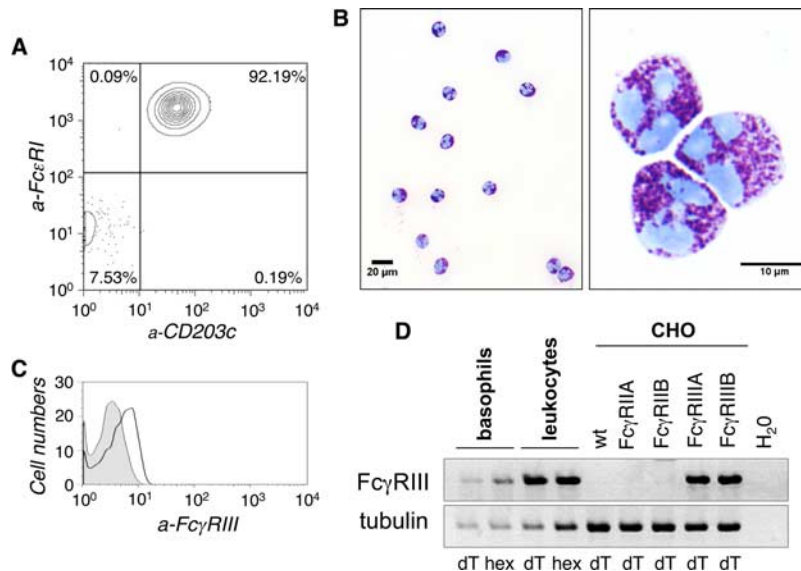
We first examined the expression of the two classes of low-affinity IgG receptors, FcγRII and FcγRIII, on basophils and, as positive and negative controls, on other white blood cells. RBC-depleted blood cells from normal donors were doubly labeled with PE-conjugated cell-type specific Abs and with FITC-conjugated anti-FcγRII (mAb FL18.26) or anti-FcγRIII (mAb 3G8) Abs, and fluorescence was analyzed in gates shown in Fig. 2*A*. As expected, neutrophils, identified by anti-CD24 Abs in gate 1, and monocytes, identified by anti-CD14 Abs in gate 2, expressed both FcγRII and FcγRIII. NK cells, identified by anti-CD56 Abs in gate 3, ex-

pressed FcγRIII, but not FcγRII, whereas B cells, identified by anti-CD19 Abs in gate 3, expressed FcγRII, but not FcγRIII. T cells, identified by anti-CD3 Abs in gate 3, expressed neither FcγRII nor FcγRIII, except a minor FcγRII⁺FcγRIII⁺ subpopulation, probably corresponding to NKT cells. As expected, basophils, identified by anti-CD203c Abs in gate 4, expressed FcγRII. Unexpectedly, basophils also expressed low but detectable levels of FcγRIII (Fig. 2*B*). Triple labeling with PE-conjugated anti-CD203c, allophycocyanin-conjugated anti-FcγRIII, and FITC-conjugated anti-IgE Abs confirmed that CD203c⁺IgE⁺ cells were indeed stained by anti-FcγRIII Abs (Fig. 2*C*).

In another set of experiments, basophils were identified in normal blood with PE-conjugated anti-CD203c and allophycocyanin-conjugated anti-FcεRI Abs, instead of anti-IgE Abs, and FcγRIII expression was assessed with another anti-FcγRIII mAb (biotinylated LNK16 and FITC-conjugated avidin, instead of FITC-conjugated 3G8). The whole CD203c⁺FcεRI⁺ basophil population was also stained with this anti-FcγRIII Ab (Fig. 2*D*).

To further confirm this finding, normal blood cells stained with PE-conjugated anti-CD203c and allophycocyanin-conjugated anti-FcεRI Abs were sorted with a FACS sorter (BD Biosciences). The sorted population contained >90% CD203c⁺FcεRI⁺ cells (Fig. 3*A*). When stained with Giemsa, sorted cells appeared as a homogenous population of granulated cells with a typical basophil

FIGURE 3. Purified basophils express Fc γ RIII and contain Fc γ RIII transcripts. **A**, CD203c⁺Fc ϵ RI⁺ cells were isolated by FACS sorting. The contour plot shows sorted cells. **B**, Cytospins were prepared and colored with Giemsa stain. The two magnifications show the homogeneity and the morphology of sorted cells, respectively. **C**, Aliquots of sorted cells were incubated with FITC-conjugated anti-Fc γ RIII Ab (mAb 3G8, open histogram) or isotype control (filled histogram) and analyzed by flow cytometry. **D**, cDNA from basophils, total blood leukocytes, and CHO transfectants was prepared either by using oligo(dT) (dT) or hexamer (hex) primers. Fc γ RIII and tubulin transcripts were detected by RT-PCR using corresponding specific primers. wt, Wild type.



morphology (Fig. 3B). They were stained with anti-Fc γ RIII mAb (Fig. 3C). They contained Fc γ RIII transcripts as shown by RT-PCR analysis with Fc γ RIII-specific primers (Fig. 3D).

These data altogether demonstrate that basophils from normal donors express not only Fc ϵ RI and Fc γ RII, but also Fc γ RIII.

Human basophils express Fc γ RIIB

Fc γ RIIA and Fc γ RIIB can be distinguished by their differential sensitivity to PI-PLC. GPI-anchored Fc γ RIIB, but not transmembrane Fc γ RIIA, are indeed cleaved by PI-PLC (33, 34). To determine which isoform(s) of Fc γ RIII is(are) expressed by basophils, RBC-depleted blood cells were therefore treated or not treated with PI-PLC and doubly labeled with PE-conjugated cell-type specific Abs and with FITC-conjugated anti-Fc γ RIII or anti-Fc γ RII Abs. PI-PLC treatment decreased the anti-Fc γ RIII staining not only of CD24⁺ neutrophils, as expected, but also of CD203c⁺ basophils (Fig. 4A). Noticeably, the mean fluorescence intensity (MFI) of anti-Fc γ RIII staining was comparably reduced (4-fold) in both cell types, and it was virtually abrogated in basophils. By contrast, PI-PLC treatment affected neither the anti-Fc γ RIII staining of CD14⁺ monocytes and CD56⁺ NK cells (Fig. 3A), which express Fc γ RIIA but not Fc γ RIIB, nor the anti-Fc γ RII staining of CD203c⁺ basophils, CD24⁺ neutrophils, and CD14⁺ monocytes (Fig. 4B). Basophils from normal donors therefore express Fc γ RIIB but not Fc γ RIIA.

Fc γ RIIB lack intracytoplasmic domain and do not associate with Fc γ . Whether they can trigger activation signals is unclear. To determine whether the engagement of Fc γ RIIB could activate basophils, CD203c expression was monitored on RBC-depleted blood cells stimulated with F(ab')₂ of anti-Fc γ RIII Abs and F(ab')₂ of RAM Abs. As positive controls, cells were also stimulated with rabbit anti-human IgE Abs. Whereas Fc ϵ RI aggregation by anti-IgE markedly up-regulated CD203c expression, no or little CD203c up-regulation was seen following Fc γ RIII aggregation (Fig. 4C). The engagement of Fc γ RIII therefore does not activate basophils or poorly, when assessed by CD203c up-regulation. This observation supports the conclusion that basophils express Fc γ RIIB, but not Fc γ RIIA.

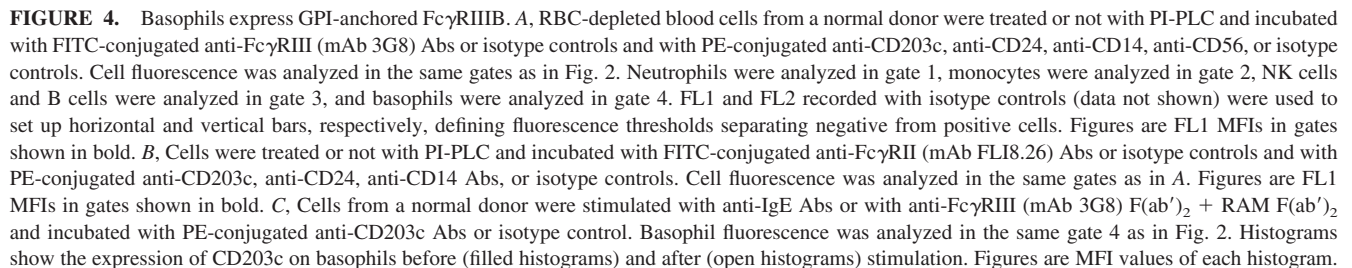
To determine whether Fc γ RIIB expression by normal basophils is a rule or an exception, RBC-depleted blood cells from 23 normal donors were doubly labeled with anti-CD203c and anti-Fc γ RIII Abs. Due to the low expression of Fc γ RIIB by basophils, the

fluorescence of cells labeled with anti-Fc γ RIII Abs partially overlaps with the fluorescence of cells labeled with isotype controls. To compare individual donors and to take into account individual background variations, Fc γ RIII expression by basophils was expressed as the percentage of CD203c⁺ blood cells stained with anti-Fc γ RIII Abs over the background staining with isotype controls (referred to as Fc γ RIII⁺ basophils) on cells from the same donor. Using this criterion, only a fraction of basophils are identified as Fc γ RIII⁺. This does not imply that Fc γ RIII is expressed by a subpopulation of basophils only. As expected, the percentage of Fc γ RIII⁺ basophils varied from donor to donor. Between 24 and 81% basophils (median, 44%) were Fc γ RIII⁺ in the population studied. Noticeably, Fc γ RIII⁺ basophils were seen in all 23 normal donors tested (Fig. 5A). Fc γ RIIB is therefore detectably expressed by at least one-fourth of blood basophils in normal individuals.

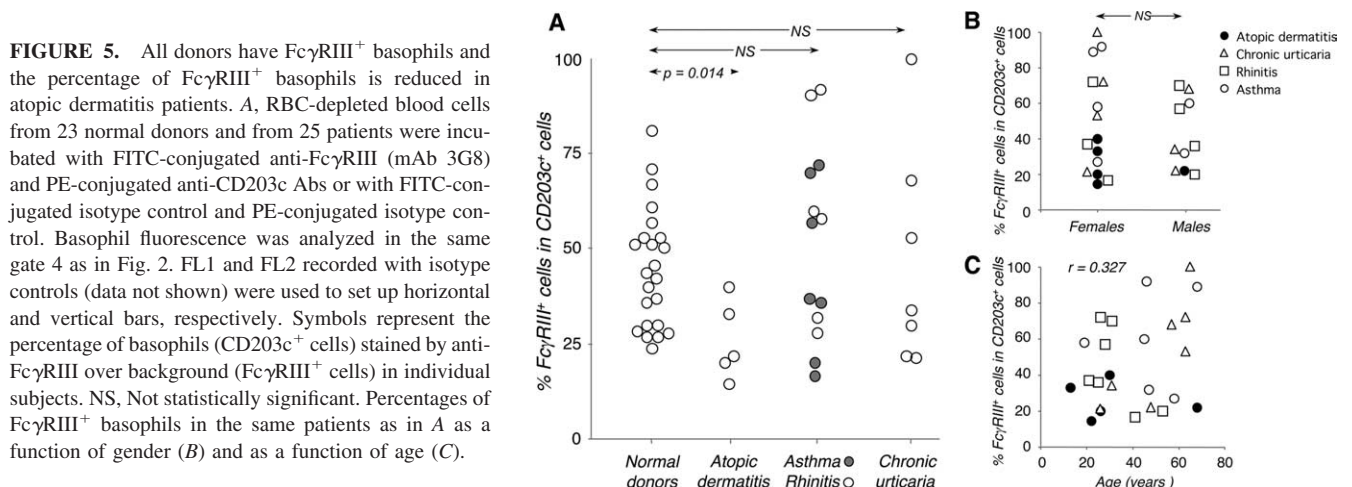
Fc γ RIIB expression is decreased on basophils from atopic dermatitis patients

We next compared Fc γ RIIB expression on basophils from normal donors and from 25 patients with diseases commonly seen in allergy practice. These were 5 patients with an allergic skin disease (atopic dermatitis), 13 patients with allergic respiratory diseases (rhinitis and asthma), and 7 patients with a nonallergic skin disease (chronic urticaria) (Table I). As in normal subjects, Fc γ RIII⁺ basophils were detected in all 25 patients. The proportion of Fc γ RIII⁺ basophils, however, varied more widely in patients than in normal donors (from 12 to 100%). It did not differ statistically from normal donors in respiratory allergy patients (median, 57%) and in chronic urticaria patients (median, 34%), but it was significantly lower ($p = 0.0142$) in atopic dermatitis patients (median, 22%) (Fig. 5A). Four of the five atopic dermatitis patients were females. The percentage of Fc γ RIII⁺ basophils was however not significantly lower in female than in male patients (Fig. 5B). On average, dermatitis patients were younger than other patients. The percentage of Fc γ RIII⁺ basophils was however not correlated with the age of the patients (Fig. 5C).

Cell activation having been reported to decrease the expression of Fc γ RIIB by neutrophils (35), we investigated whether basophil activation might account for the decreased Fc γ RIIB expression seen in atopic dermatitis patients. Fc γ RIIB and CD203c expression were examined first on basophils from eight normal donors



FcγRIIIB expression markedly dropped on basophils from four of the five donors with a high expression, and decreased on basophils from the three donors with a weak expression (Fig. 6A; median MFI decrease, 2-fold). Anti-IgE-induced basophil activation therefore correlates with a decreased FcγRIIIB expression. On the basis



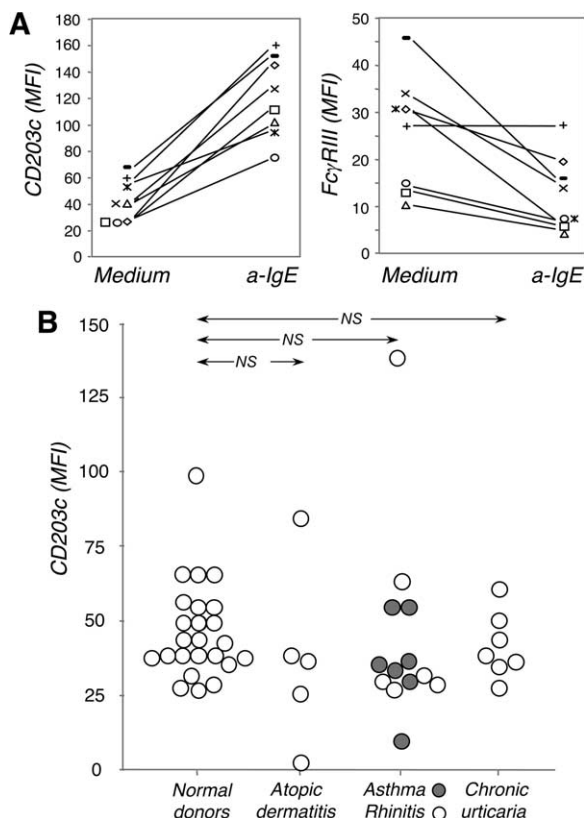


FIGURE 6. Fc γ RIII expression is decreased upon anti-IgE-induced basophil activation, but the basophils of atopic dermatitis patients do not have an activated phenotype. *A*, Cells from eight normal donors were stimulated with anti-IgE Abs or without and incubated with PE-conjugated anti-CD203c and FITC-conjugated anti-Fc γ RIII (mAb 3G8) Abs or with PE-conjugated isotype control and FITC-conjugated isotype control. Basophil fluorescence was analyzed in the same gate 4 as in Fig. 2. MFI values of FL2 (anti-CD203c) and FL1 (anti-Fc γ RIII) were recorded in the same stimulated and nonstimulated cells. *B*, CD203c expression in the same normal donors and patients as in Fig. 5*A*. NS, Not statistically significant.

of this finding, we hypothesized that basophils might be constitutively activated in atopic dermatitis patients, and we examined the intensity of anti-CD203c staining in the three groups of patients.

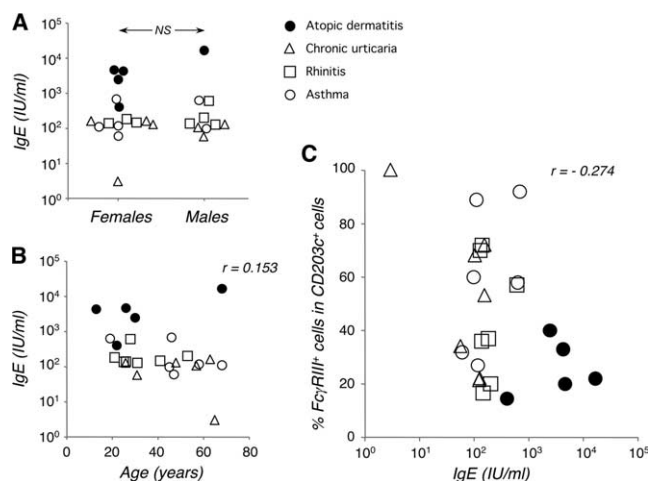


FIGURE 7. The percentage of Fc γ RIII⁺ basophils is not correlated with serum IgE. Serum IgE levels in the same patients as in Fig. 5*A* as a function of gender (*A*) and as a function of age (*B*). *C*, Percentages of Fc γ RIII⁺ basophils in the same patients as in Fig. 5*A* as a function of serum IgE concentration. NS, Not statistically significant; *r*, Pearson's correlation coefficient.

The MFI of anti-CD203c staining was not significantly different in atopic dermatitis patients, compared with normal donors (Fig. 6*B*).

A hallmark of atopic dermatitis is a high serum IgE level and, indeed, the serum concentration of IgE was markedly higher in atopic dermatitis patients (median, 4273 IU/ml) than in allergic respiratory disease patients (median, 147 IU/ml) and in chronic urticaria patients (median, 126 IU/ml; Table I). IgE concentration was correlated neither with the gender (Fig. 7*A*) nor with the age of the patients (Fig. 7*B*). The percentage of Fc γ RIII⁺ basophils was not correlated with serum IgE levels either (Fig. 7*C*).

Discussion

Results reported here challenge two widely accepted notions. First, human basophils are considered as CD16-negative cells (13–15). We show here that blood basophils detectably expressed Fc γ RIII in all donors, whether normal individuals or patients. Second, the GPI-anchored Fc γ RIIIB is considered to be solely expressed by human neutrophils (23). We show that Fc γ RIIIB is also expressed by basophils. Interestingly, we also show that the percentage of Fc γ RIIIB-expressing basophils is lower in atopic dermatitis patients than in normal donors. These findings may have practical, fundamental, and clinical implications.

Fc γ RIII are typically expressed by blood neutrophils, monocytes, NK, and NKT cells, but not by B cells, T cells, and basophils. We found that at least 24% and up to 81% basophils from normal donors and at least 12% and up to 100% basophils from allergic (and nonallergic) patients were detectably labeled by anti-Fc γ RIII Abs. Fc γ RIII were detected by direct and indirect immunofluorescence, using two anti-Fc γ RIII mAbs, on basophils identified in blood from normal donors as CD203c⁺IgE⁺ or as CD203c⁺Fc ϵ RI⁺ cells and on CD203c⁺Fc ϵ RI⁺ sorted cells that had a typical basophil morphology. Although 92% sorted cells only were doubly labeled with anti-CD203c and anti-Fc ϵ RI Abs, all had a basophil morphology when stained with Giemsa. A likely explanation is that altered cells or debris accounted for the 8% unlabeled cells. Fc γ RIII sequences were amplified by RT-PCR in cDNA transcribed from RNA extracted from sorted basophils. The primers used were Fc γ RIII specific as they amplified sequences in cDNA from CHO transfectants expressing Fc γ RIIIA or Fc γ RIIIB, but not from transfectants expressing Fc γ RIIA or Fc γ RIIB. Fc γ RIII sequences were amplified both in cDNA transcribed from total RNA and in cDNA transcribed from poly(A) RNA extracted from sorted basophils.

Several reasons may explain why Fc γ RIII was not previously observed on basophils. One is that studies in which basophils were first phenotyped were done at a time when human Fc γ R isoforms had not yet been all identified and with mAbs whose specificity for Fc γ R subtypes was unclear (36–38). Another reason is the much lower expression of Fc γ RIII by basophils than by other Fc γ RIII⁺ blood cells. MFI values of anti-Fc γ RIII staining were indeed 10- to 100-fold lower for basophils than for monocytes, NK cells, or neutrophils. Techniques with a relatively low sensitivity, which would be sufficient to brightly label these cells, may not or hardly label basophils. One may have interpreted a weak basophil labeling as background labeling and have neglected it. Finally, Fc γ RIII may not have been seen in negatively sorted basophil preparations because Fc γ RIII⁺ basophils were discarded. Basophil enrichment techniques use magnetic beads and a mixture of mAbs against membrane molecules that are not expressed by basophils. As basophils were described to express CD13, CD22, CD32, CD33, CD123 and CD203c, but not CD2, CD3, CD14, CD15, CD16, CD19, CD21, CD64, and HLA-DR (13–15), negative selection procedures used to enrich basophils from white blood cells include

anti-CD16, i.e., anti-Fc γ RIII (13, 39), among other mAbs. Although Fc γ RIII expression is low on basophils, one cannot exclude that enrichment procedures in which CD16⁺ cells are discarded may remove a significant proportion of basophils. This would introduce a bias in sorted basophils. It may also decrease the yield of these techniques.

No mAb is available that discriminates Fc γ RIIIA from Fc γ RIIIB. On the basis of the differential sensitivity of Fc γ RIIIA and Fc γ RIIIB to PI-PLC, we however found that basophils express the GPI-anchored Fc γ RIIIB but, apparently, not the transmembrane FcR γ -associated Fc γ RIIIA. PI-PLC treatment of blood cells indeed reduced similarly the staining of basophils and neutrophils, but not of monocytes or NK cells, by anti-Fc γ RIII, and had no detectable effect on the staining of any blood cells, including basophils, by anti-Fc γ RII. The anti-Fc γ RIII staining of basophils was reduced below the background level in PI-PLC-treated cells, suggesting that basophils do not express Fc γ RIIIA. The expression of Fc γ RIIIB is therefore not restricted to neutrophils, as previously thought. Noticeably, intracellular Fc γ RIIIB was reported, in one article (40), in normal eosinophils, suggesting the possibility that all three types of polymorphonuclear granulocytes may express the *Fcgr3b* gene.

The biological functions of Fc γ RIIIB are poorly understood. When expressed in transfectants and aggregated by appropriate plurivalent ligands, Fc γ RIIIB did not trigger activation signals (41). Fc γ RIIIB aggregation was, however, reported to trigger the production of H₂O₂ and an increased intracellular Ca²⁺ concentration in neutrophils (42). Neutrophil Fc γ RIIIB were also found to trigger degranulation (43) and to activate Tec (44) and Syk (45) protein tyrosine kinases. We failed to induce a significant CD203c up-regulation when aggregating Fc γ RIIIB on basophils under similar conditions. CD203c up-regulation is associated with exocytosis (26). Whether the engagement of Fc γ RIIIB can induce responses of basophils other than degranulation, e.g., cytokine synthesis/secretion, remains to be investigated. In any case, our finding that basophils do not degranulate in response to Fc γ RIII engagement supports the conclusion that basophils do not express Fc γ RIIIA.

Like other GPI-anchored molecules, Fc γ RIIIB constitutively resides in cholesterol- and sphingolipid-rich plasma membrane microdomains or lipid rafts (45, 46). Signaling molecules, including Src family protein tyrosine kinases (47) and the transmembrane adapter LAT (48), are also located in lipid rafts, and the aggregation of lipid raft-associated gangliosides was reported to trigger activation signals (49). Besides, Fc γ RIIIB has been proposed to synergize with activating FcRs (43) by facilitating the binding of IgG immune complexes to other Fc γ Rs on the same cell (45, 50). Alternatively, Fc γ RIIIB could compete with other Fc γ Rs for immune complex binding. Whether Fc γ RIIIB may enhance or decrease cell signaling by other basophil Fc γ Rs needs to be determined. The expression of several low-affinity IgG receptors with different signaling properties may indeed explain why no clear-cut conclusion was drawn regarding the ability of IgG immune complexes to activate basophils (51).

Interestingly, the percentage of Fc γ RIIIB⁺ basophils was significantly lower in atopic dermatitis patients than in normal donors, but not in chronic urticaria patients, in allergic rhinitis patients, or in allergic asthma patients. A decreased expression of Fc γ RIIIB by basophils was therefore observed in an allergic skin disease, but neither in a non-allergic inflammatory skin disease nor in two allergic respiratory diseases. Noticeably, Fc γ RIIIB expression was not decreased on neutrophils from atopic dermatitis patients (data not shown), suggesting that Fc γ RIIIB down-regulation selectively affects basophils. The expression of Fc γ RIIIB was previously re-

ported to be down-regulated on activated neutrophils (35) and Fc γ RIIIB down-regulation was proposed to result from the cleavage of the receptor by proteases released and/or activated during neutrophil activation (52). We found that the expression of Fc γ RIIIB was also down-regulated on activated basophils, and similar mechanisms may operate in the two cell types. Basophils may indeed release and/or activate proteases when activated. Although mast cell proteases are well characterized and used as mast cell type-specific markers (53), basophil proteases are poorly known. Murine basophils were however described to express mMCP-8. This protease is related to T cell granzymes and to the neutrophil cathepsin G, rather than to mast cell tryptases or chymases (54). Fc γ RIIIB down-regulation was however not correlated with CD203c up-regulation on basophils from atopic dermatitis patients, suggesting that it was not associated with a possible chronic basophil activation. The percentages of Fc γ RIIIB⁺ basophils were not correlated either with serum IgE levels which were markedly elevated in atopic dermatitis patients.

IgE-bearing APCs, T cells, mast cells, eosinophils, and keratinocytes concur to generate atopic dermatitis lesions (55). Interestingly, basophils were observed in skin biopsies after allergen challenge and in skin lesions from atopic dermatitis patients (56). The biological significance of the decreased frequency of Fc γ RIII⁺ basophils in atopic dermatitis patients is not known. Whether it is a cause or a consequence in this disease and whether it has pathological consequences need to be investigated. It nevertheless supports the idea that, although a skin disease, atopic dermatitis is a systemic disease rather than a disease of the skin.

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Disclosures

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