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

Nihad Meknache, Friederike Jönsson, Jérôme Laurent, Marie-Thérèse Guinnepain and Marc Daëron

*J Immunol* 2009; 182:2542-2550; doi: 10.4049/jimmunol.0801665

http://www.jimmunol.org/content/182/4/2542
Human Basophils Express the Glycosylphosphatidylinositol-Anchored Low-Affinity IgG Receptor FcγRIIIB (CD16B)

Nihad Meknache,2*† Friederike Jönsson,2*† Jérôme Laurent,‡ Marie-Thérèse Guinnepain,‡ and Marc Daeüron3*†

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 (FcγRIIAB and FcγRIIB). FcγRIIA, FcγRIIC, and FcγRIIIA are activating receptors, FcγRIIB are inhibitory receptors, FcγRIIIA 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 FcγRIIIA transcripts and express FcγRIIIB, but not FcγRIIIA, which were detected on 24–81% basophils from normal donors and on 12–100% basophils from patients. Noticeably, the proportion of FcγRIIIB+ 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 FcγRIIIA 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 FcγRIIIB is exclusively expressed by neutrophils. They suggest that a proportion of basophils may be lost during enrichment procedures in which FcγRIIIA+ 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 FcεRIγ and FcεRIβ, the two FcεRI 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 FcγRII, which also associate with FcγRI and FcγRIβ in these cells. FcγRIIIA activate mouse mast cells, both in vitro (6) and in vivo (7). Whether mouse basophils express FcγRIIIA 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 FcγRIIAC (9), which do not associate with FcγRI or FcγRIβ, but which contain an ITAM in their own intracytoplasmic domain. FcγRIIAC 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, FcγRIIC, 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

*Institut Pasteur, Département d’Immunologie, Unité d’Allergologie Moléculaire et Cellulaire, Paris; †Institut National de la Santé et de la Recherche Médicale, Unité 760, Paris; and ‡Consultation d’Allergologie, Centre Médical de l’Institut Pasteur, Paris, France

Received for publication May 23, 2008. Accepted for publication December 17, 2008.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 This work was supported by the Institut Pasteur, the Institut National de la Santé et de la Recherche Médicale, the Agence Nationale pour la Recherche, and the Fondation pour la Recherche Médicale Program Défis de la Recherche en Allergologie. N.M. was financially supported by the Fondation pour la Recherche Médicale et le Institut Pasteur; F.J. was financially supported by the Agence Nationale pour la Recherche.

2 N.M. and F.J. contributed equally.

3 Address correspondence and reprint requests to Dr. Marc Daeüron, Unité d’Allergologie Moléculaire et Cellulaire, Bâtiment Metchnikoff, Institut Pasteur, 25 rue du Docteur Roux, 75015 Paris, France. E-mail address: daeron@pasteur.fr
Fcα is expressed by monocytes (19), NK cells (20), and NKT cells (21). Similarly, FcγRII is constitutively expressed by and up-regulated upon basophil activation. Using CD203c as a marker, we undertook an investigation of FcγRII expression and its increased expression as an indicator of basophil activation and its increased expression as an indicator of blood basophils (14, 26). It is constitutively expressed and up-regulated upon basophil activation (17) 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 techniques are not without inconvenience. Basophils may be altered (e.g., activated) by positive selection procedures while some techniques are not without inconvenience. Basophils may be altered (e.g., activated) by positive selection procedures while some techniques are not without inconvenience.

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 facs (e.g., elu-

tration) 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 FcγR expression 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 FcγRIIB, but not FcγRIIA, that FcγRIIB is detectably expressed by at least one-fourth of basophils in all normal donors, and that the percentage of FcγRIIB+ 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 Allergology 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 I. Patients included in this study

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (years)</th>
<th>Sex</th>
<th>Disease</th>
<th>Allergens</th>
<th>Severity</th>
<th>Total IgE (kU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>21</td>
<td>F</td>
<td>Atopic dermatitis</td>
<td>Polysensitized</td>
<td>Moderate</td>
<td>402</td>
</tr>
<tr>
<td>2</td>
<td>26</td>
<td>F</td>
<td>Atopic dermatitis</td>
<td>Polysensitized</td>
<td>Moderate</td>
<td>4620</td>
</tr>
<tr>
<td>3</td>
<td>68</td>
<td>M</td>
<td>Atopic dermatitis</td>
<td>Polysensitized</td>
<td>Moderate</td>
<td>16556</td>
</tr>
<tr>
<td>4</td>
<td>30</td>
<td>F</td>
<td>Atopic dermatitis</td>
<td>Polysensitized</td>
<td>Moderate</td>
<td>2545</td>
</tr>
<tr>
<td>5</td>
<td>13</td>
<td>F</td>
<td>Atopic dermatitis</td>
<td>Polysensitized</td>
<td>Moderate</td>
<td>2473</td>
</tr>
<tr>
<td>6</td>
<td>31</td>
<td>M</td>
<td>Chronic urticaria</td>
<td>None</td>
<td>Moderate</td>
<td>57</td>
</tr>
<tr>
<td>7</td>
<td>57</td>
<td>M</td>
<td>Chronic urticaria</td>
<td>None</td>
<td>Moderate</td>
<td>105</td>
</tr>
<tr>
<td>8</td>
<td>26</td>
<td>F</td>
<td>Chronic urticaria</td>
<td>None</td>
<td>Moderate</td>
<td>126</td>
</tr>
<tr>
<td>9</td>
<td>26</td>
<td>F</td>
<td>Chronic urticaria</td>
<td>None</td>
<td>Dermographia</td>
<td>266</td>
</tr>
<tr>
<td>10</td>
<td>48</td>
<td>M</td>
<td>Chronic urticaria</td>
<td>None</td>
<td>Moderate</td>
<td>128</td>
</tr>
<tr>
<td>11</td>
<td>63</td>
<td>F</td>
<td>Chronic urticaria</td>
<td>None</td>
<td>Moderate</td>
<td>158</td>
</tr>
<tr>
<td>12</td>
<td>65</td>
<td>F</td>
<td>Chronic urticaria</td>
<td>None</td>
<td>Moderate</td>
<td>3</td>
</tr>
<tr>
<td>13</td>
<td>31</td>
<td>M</td>
<td>Rhinitis</td>
<td>Pollen (grass, birch)</td>
<td>Moderate</td>
<td>128</td>
</tr>
<tr>
<td>14</td>
<td>53</td>
<td>M</td>
<td>Rhinitis</td>
<td>Pollen (grass, birch)</td>
<td>Moderate</td>
<td>200</td>
</tr>
<tr>
<td>15</td>
<td>41</td>
<td>F</td>
<td>Rhinitis</td>
<td>Mites, cat</td>
<td>Moderate</td>
<td>146</td>
</tr>
<tr>
<td>16</td>
<td>36</td>
<td>F</td>
<td>Rhinitis</td>
<td>Pollen (grass, birch, mites)</td>
<td>Moderate</td>
<td>140</td>
</tr>
<tr>
<td>17</td>
<td>25</td>
<td>M</td>
<td>Rhinitis</td>
<td>Mites, cat</td>
<td>Moderate</td>
<td>137</td>
</tr>
<tr>
<td>18</td>
<td>28</td>
<td>M</td>
<td>Rhinitis</td>
<td>Pollen (grass)</td>
<td>Moderate</td>
<td>609</td>
</tr>
<tr>
<td>19</td>
<td>21</td>
<td>F</td>
<td>Rhinitis</td>
<td>Pollen (grass, birch)</td>
<td>Moderate</td>
<td>183</td>
</tr>
<tr>
<td>20</td>
<td>19</td>
<td>F</td>
<td>Asthma</td>
<td>Pollen (grass)</td>
<td>Persistent/moderate</td>
<td>628</td>
</tr>
<tr>
<td>21</td>
<td>68</td>
<td>F</td>
<td>Asthma</td>
<td>Mites</td>
<td>Persistent/moderate</td>
<td>110</td>
</tr>
<tr>
<td>22</td>
<td>46</td>
<td>F</td>
<td>Asthma</td>
<td>Pollen (grass, birch, cat)</td>
<td>Persistent/moderate</td>
<td>684</td>
</tr>
<tr>
<td>23</td>
<td>45</td>
<td>M</td>
<td>Asthma</td>
<td>Mites</td>
<td>Persistent/moderate</td>
<td>98</td>
</tr>
<tr>
<td>24</td>
<td>58</td>
<td>F</td>
<td>Asthma</td>
<td>Pollen (grass, mites)</td>
<td>Persistent/moderate</td>
<td>117</td>
</tr>
<tr>
<td>25</td>
<td>47</td>
<td>M</td>
<td>Asthma</td>
<td></td>
<td>Persistent/moderate</td>
<td>60</td>
</tr>
</tbody>
</table>

* F. Female; M. male.

**a** Associated with autoimmune thyroiditis.
anti-FcεRI 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 DakoCytomation. 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-FcεRI and PE-conjugated anti-CD203c Abs and separated using a MoFlo sorter (DakoCytomation). To assess the purity of sorted cells, cytospins 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 FcγRIIA, FcγRIIB, or FcγRIIB (32). cDNA was produced using Superscript II Reverse Transcriptase (Invitrogen) either with oligo(dT) or random hexamers. The following primers (32). cDNA was produced using Superscript II Reverse Transcriptase (Invitrogen) either with oligo(dT) or random hexamers. The following primers

[Table II. Abs used for immunofluorescence analysis]

<table>
<thead>
<tr>
<th>Label</th>
<th>Isotype</th>
<th>Ab (clone)</th>
<th>Isotype Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>FITC</td>
<td>Mouse IgG1</td>
<td>Anti-CD16 (3G8)</td>
<td>MOPC-21</td>
</tr>
<tr>
<td></td>
<td>Mouse IgG2b</td>
<td>Anti-CD32 (FL8.26)</td>
<td>27–35</td>
</tr>
<tr>
<td></td>
<td>Goat IgG</td>
<td>Anti-human IgE</td>
<td>Normal goat IgG</td>
</tr>
<tr>
<td>PE</td>
<td>Mouse IgG1</td>
<td>Anti-CD56 (B159)</td>
<td>MOPC-21</td>
</tr>
<tr>
<td></td>
<td>Mouse IgG1</td>
<td>Anti-CD19 (HB19)</td>
<td>MOPC-21</td>
</tr>
<tr>
<td></td>
<td>Mouse IgG1</td>
<td>Anti-CD203c (97A6)</td>
<td>679.1Mc7</td>
</tr>
<tr>
<td></td>
<td>Mouse IgG2a</td>
<td>Anti-CD14 (MSE2)</td>
<td>G155–178</td>
</tr>
<tr>
<td></td>
<td>Mouse IgG2a</td>
<td>Anti-CD3 (HIT3a)</td>
<td>G155–178</td>
</tr>
<tr>
<td></td>
<td>Mouse IgG2a</td>
<td>Anti-CD24 (ML5)</td>
<td>G155–178</td>
</tr>
<tr>
<td>Allophycocyanin</td>
<td>Mouse IgG1</td>
<td>Anti-CD16 (3G8)</td>
<td>MOPC-21</td>
</tr>
<tr>
<td></td>
<td>Mouse IgG2b</td>
<td>Anti-FcεRI (AER-37)</td>
<td>27–35</td>
</tr>
<tr>
<td>Biotin</td>
<td>Mouse IgG1</td>
<td>Anti-CD16 (LNK16)</td>
<td>—</td>
</tr>
</tbody>
</table>

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

![Figure 1](http://www.jimmunol.org/Downloadedfromhttp://www.jimmunol.org/)

**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.
Results

**Human basophils express FcγRIII**

CD203c is a specific marker of human basophils (14, 26). Blood basophils also express FcγRI, 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 ± 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. 2A. 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, expressed FcγRII, but not FcγRIII, 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⁺FcyRIII⁺ 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 FcyRIII (Fig. 2B). Triple labeling with PE-conjugated anti-CD203c, allogeneic-conjugated anti-FcγRII, and FITC-conjugated anti-IgE Abs confirmed that CD203c⁺ IgE⁺ cells were indeed stained by anti-FcγRIII Abs (Fig. 2C).

In another set of experiments, basophils were identified in normal blood with PE-conjugated anti-CD203c and allogeneic-conjugated anti-FcγRII 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⁺Fcr⁺ basophil population was also stained with this anti-FcγRIII Ab (Fig. 2D).

To further confirm this finding, normal blood cells stained with PE-conjugated anti-CD203c and allogeneic-conjugated anti-FcγRII Abs were sorted with a FACSorter (BD Biosciences). The sorted population contained >90% CD203c⁺Fcr⁺ cells (Fig. 3A). When stained with Giemsa, sorted cells appeared as a homogeneous population of granulated cells with a typical basophil
HUMAN BASOPHILS EXPRESS FcγRIII (CD16B)

**FIGURE 3.** Purified basophils express FcγRIII and contain FcγRIII transcripts. A, CD203c⁺ FcγRII⁺ 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-FcγRIII 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. FcγRIII and tubulin transcripts were detected by RT-PCR using corresponding specific primers. wt, Wild type.

**Human basophils express FcγRIII**

FcγRIIIA and FcγRIIIB can be distinguished by their differential sensitivity to PI-PLC. GPI-anchored FcγRIIIA, but not transmembrane FcγRIIIA, are indeed cleaved by PI-PLC (33, 34). To determine which isofrom(s) of FcγRIII is(are) expressed by basophils, RBC-depleted blood cells were therefore treated or not with PI-PLC and doubly labeled with PE-conjugated cell-type specific Abs and with FITC-conjugated anti-FcγRIII or anti-FcγRII Abs. PI-PLC treatment decreased the anti-FcγRIII staining not only of CD24⁺ neutrophils, as expected, but also of CD203c⁺ basophils (Fig. 4A). Noticeably, the mean fluorescence intensity (MFI) of anti-FcγRIII 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-FcγRII staining of CD14⁺ monocytes and CD56⁺ NK cells (Fig. 3A), which express FcγRIIIA but not FcγRIIIB, nor the anti-FcγRII staining of CD203c⁺ basophils, CD24⁺ neutrophils, and CD14⁺ monocytes (Fig. 4B). Basophils from normal donors therefore express FcγRIIIB but not FcγRIIIA.

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

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

**FcγRIIIB expression is decreased on basophils from atopic dermatitis patients**

We next compared FcγRIIIB expression on basophils from normal donors and to 25 patients with diseases commonly seen in allergy and asthma. 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, FcγRIII⁺ basophils were detected in all 25 patients. The proportion of FcγRIII⁺ basophils, however, varied more widely in patients than in normal donors (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 FcγRIII⁺ 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 FcγRIII⁺ basophils was however not correlated with the age of the patients (Fig. 5C).

Cell activation having been reported to decrease the expression of FcγRIIIB by neutrophils (35), we investigated whether basophil activation might account for the decreased FcγRIIIB expression seen in atopic dermatitis patients. FcγRIIIB and CD203c expression were examined first on basophils from eight normal donors...
before and after stimulation with anti-IgE Abs. The MFI of anti-CD203c staining increased similarly on basophils from all eight donors following anti-IgE stimulation (median MFI increase, 2.8-fold). The MFI of anti-FcγRIII staining differed on nonstimulated basophils from the same donors. Following anti-IgE stimulation, FcγRIIB 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 FcγRIIB expression. On the basis

FIGURE 4. Basophils express GPI-anchored FcγRIIB. A, RBC-depleted blood cells from a normal donor were treated or not with PI-PLC and incubated with FITC-conjugated anti-FcγRIII (mAb 3G8) Abs or isotype controls and with PE-conjugated anti-CD203c, anti-CD24, anti-CD14, anti-CD56, or isotype controls. Cell fluorescence was analyzed in the same gates as in Fig. 2. Neutrophils were analyzed in gate 1, monocytes were analyzed in gate 2, NK cells and B 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. Figures are FL1 MFIs in gates shown in bold. B, Cells were treated or not with PI-PLC and incubated with FITC-conjugated anti-FcγRIII (mAb FL18.26) Abs or isotype controls and with PE-conjugated anti-CD203c, anti-CD24, anti-CD14 Abs, or isotype controls. Cell fluorescence was analyzed in the same gates as in A. Figures are FL1 MFIs in gates shown in bold. C, Cells from a normal donor were stimulated with anti-IgE Abs or with anti-FcγRIII (mAb 3G8) F(ab')2 + RAM F(ab')2 and incubated with PE-conjugated anti-CD203c Abs or isotype control. Basophil fluorescence was analyzed in the same gate 4 as in Fig. 2. Histograms show the expression of CD203c on basophils before (filled histograms) and after (open histograms) stimulation. Figures are MFI values of each histogram.

FIGURE 5. All donors have FcγRIII⁺ basophils and the percentage of FcγRIII⁺ basophils is reduced in atopic dermatitis patients. A, RBC-depleted blood cells from 23 normal donors and from 25 patients were incubated with FITC-conjugated anti-FcγRIII (mAb 3G8) and PE-conjugated anti-CD203c Abs or with FITC-conjugated isotype control and PE-conjugated isotype control. Basophil fluorescence was analyzed in the same gate 4 as in Fig. 2. FL1 and FL2 recorded with isotype controls (data not shown) were used to set up horizontal and vertical bars, respectively. Symbols represent the percentage of basophils (CD203c⁺ cells) stained by anti-FcγRIII over background (FcγRIII⁻ cells) in individual subjects. NS, Not statistically significant. Percentages of FcγRIII⁺ basophils in the same patients as in A as a function of gender (B) and as a function of age (C).
The MFI of anti-CD203c staining was not significantly different in atopic dermatitis patients, compared with normal donors (Fig. 6B).

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. 7A) nor with the age of the patients (Fig. 7B). The percentage of FcγRIII* basophils was not correlated with serum IgE levels either (Fig. 7C).

FIGURE 7. The percentage of FcγRIII* basophils is not correlated with serum IgE. Serum IgE levels in the same patients as in Fig. 5A as a function of gender (A) and as a function of age (B). C, Percentages of FcγRIII* basophils in the same patients as in Fig. 5A as a function of serum IgE concentration. NS, Not statistically significant; r, Pearson’s correlation coefficient.
anti-CD16, i.e., anti-FcγRIII (13, 39), among other mAbs. Although FcγRIII 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 FcγRIIIA from FcγRIIIB. On the basis of the differential sensitivity of FcγRIIIA and FcγRIIIB to PI-PLC, we however found that basophils express the GPI-anchored FcγRIIIIB but, apparently, not the transmembrane Fcγ-γ-associated FcγRIIIA. PI-PLC treatment of blood cells indeed reduced similarly the staining of basophils and neutrophils, but not of monocytes or NK cells, by anti-FcγRIII, and had no detectable effect on the staining of any blood cells, including basophils, by anti-FcγRII. The anti-FcγRII staining of basophils was reduced below the background level in PI-PLC-treated cells, suggesting that basophils do not express FcγRIIIA. The expression of FcγRIIIB is therefore not restricted to neutrophils, as previously thought. Noticeably, intracellular FcγRIIIB was reported, in one article (40), in normal eosinophils, suggesting the possibility that all three types of polymorphonuclear granulocytes may express the FcγRIIb gene.

The biological functions of FcγRIIIB are poorly understood. When expressed in transfectants and aggregates by appropriate plurivalent ligands, FcγRIIIB did not trigger activation signals (41). FcγRIIIB aggregation was, however, reported to trigger the production of H2O2 and an increased intracellular Ca2+ concentration in neutrophils (42). Neutrophil FcγRIIIB 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 FcγRIIIB on basophils under similar conditions. CD203c up-regulation is associated with exocytosis (26). Whether the engagement of FcγRIIIB can induce responses of basophils other than degranulation, e.g., cytokine synthesis/release, remains to be investigated. In any case, our finding that basophils do not degranulate in response to FcγRIIIB engagement supports the conclusion that basophils do not express FcγRIIIA.

Like other GPI-anchored molecules, FcγRIIIB constitutively resides in cholesterol- and sphingolipid-rich 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, FcγRIIIB has been proposed to synergize with activating FcRs (43) by facilitating the binding of IgG immune complexes to other FcγRs on the same cell (45, 50). Alternatively, FcγRIIIB could compete with other FcγRs for immune complex binding. Whether FcγRIIIB may enhance or decrease cell signaling by other basophil FcγRs 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 FcγRIIIB+ 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 FcγRIIIB 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, FcγRIIIB expression was not decreased on neutrophils from atopic dermatitis patients (data not shown), suggesting that FcγRIIIB down-regulation selectively affects basophils. The expression of FcγRIIIB was previously reported to be down-regulated on activated neutrophils (35) and FcγRIIIB 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 FcγRIIIB 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). FcγRIIIB 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 FcγRIIIB+ 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 FcγRIIIB+ 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.

Acknowledgments
We are grateful to A.-S. Le Guern (Centre Médical de l’Institut Pasteur, Paris, France) for serum IgE measurements, Dr. Ana Cumano (Unité du Développement des Lymphocytes, Institut Pasteur) for generous help and expertise in cell sorting, and the Flow Cytometry Platform Facility and the Dynamic Imaging Platform (Imagopole, Institut Pasteur).

Disclosures
The authors have no financial conflict of interest.

References