

Michel Joseph¹,
 Abdelillah Soussi Gounni²,
 Jean-Pierre Kusnier²,
 Han Vorng¹,
 Marika Sarfati³,
 Jean-Pierre Kinet⁴,
 André-Bernard Tonnel¹,
 André Capron² and
 Monique Capron²

¹ Unité INSERM 416, Institut Pasteur, Lille, France

² Unité INSERM 167, Institut Pasteur, Lille, France

³ Centre de Recherches en Allergie, Hôpital Notre-Dame, Montréal, Québec, Canada;

⁴ Harvard Medical School, Beth Israel Hospital, Boston, USA

Expression and functions of the high-affinity IgE receptor on human platelets and megakaryocyte precursors

Platelets can be activated by IgE and are therefore involved in IgE-mediated effector mechanisms against parasites and in allergic disorders. Here we show that, besides the low-affinity IgE receptor (FcεRII/CD23), platelets express the high-affinity receptor for IgE (FcεRI). Flow cytometry analysis revealed the existence of surface FcεRI on platelets with a large heterogeneity among individual donors, and a low proportion of platelets co-expressing FcεRI and FcεRII/CD23. Northern hybridization and reverse transcription polymerase chain reaction confirmed the presence of mRNA encoding the α, β and γ chains of FcεRI in platelets and in their megakaryocytic precursors. Cross-linking of FcεRI with monoclonal antibody (mAb) to α chain using either the whole molecule or F(ab')₂ triggered platelet cytotoxicity for *Schistosoma mansoni* larvae. Anti-FcεRII/CD23 mAb significantly inhibited IgE- or FcεRI-mediated cytotoxicity, indicating down-regulatory effects of FcεRII/CD23 on FcεRI-dependent functions. These results demonstrate functional properties for FcεRI on platelets and indicate unsuspected interactions between the low- and the high-affinity IgE receptors.

1 Introduction

Investigations of immunological functions of platelets have shown that these were reactive to IgE and could either participate in IgE-mediated protective immunity against helminth and protozoan parasites [1–4], or release proinflammatory mediators in the immediate and late-phase reactions of allergic disorders [5–7]. These IgE-mediated functions were first associated with the presence of a low-affinity receptor of IgE (FcεRII/CD23), as demonstrated by flow cytometry using anti-CD23 monoclonal antibodies (mAb) [8, 9]. The binding of IgE to platelets and their cross-linking by anti-IgE antibody or by specific antigens (either parasite antigens or allergens) resulted in a stimulation process followed by the generation of mediators which are cytotoxic for parasite larvae and by the release of free radicals [8]. Polyclonal and monoclonal anti-CD23 antibodies were able to significantly inhibit both IgE-dependent stimulation and binding of radiolabeled IgE to platelets at a level similar to that achieved by a 50-fold excess of unlabeled IgE [8]. It was therefore concluded that IgE could bind to platelets and induce cytotoxic functions through the FcεRII/CD23. These properties were shared by other inflammatory cells, such as mononuclear phagocytes and eosinophils, and this led to the concept of a dual function of IgE receptors, potentially involved in protective immunity to parasites and in allergic disorders [10].

[I 16729]

Received February 5, 1997; in revised form May 22, 1997; accepted May 28, 1997.

Present address: A.S. Gounni, Meakins Christie Laboratories, Montréal, Quebec, Canada

Correspondence: Michel Joseph, INSERM U 416, Institut Pasteur, B.P. 245, F-59019 Lille, France
 Fax: +33-320-877-345

Key words: Human platelet / FcεRI / CD 23 / Megakaryocyte / *Schistosoma mansoni*

In allergic manifestations, the crucial role of IgE has been mainly associated with the high-affinity receptor for IgE (or FcεRI), constitutively expressed by mast cells and basophils (reviewed in [11]). The recent demonstration that other cell populations, such as Langerhans' cells, eosinophils and monocytes, could also express FcεRI [12–14] and that, in the case of eosinophils, FcεRI could participate in their dual functions, including anti-parasite cytotoxicity and release of mediators [13], led us to investigate its expression and role in platelet functions.

2 Materials and methods

2.1 Immunological reagents

IgE receptors were identified through anti-FcεRI mouse mAb 15-1 [15] specific for the extracytoplasmic α chain containing the IgE-binding domain, anti-FcεRII/CD23 mouse mAb 135 [16] and polyclonal rabbit antiserum (Rb55 [17], kindly provided by Dr. Jean-Yves Bonnefoy, Glaxo Institute, Geneva) against the IgE-binding domain of B lymphocyte CD23. Fluorescent labeling was carried out with FITC- or PE-conjugated goat IgG anti-mouse IgG1, FITC-conjugated goat anti-rabbit IgG (Jackson ImmunoResearch Laboratories, West Grove, PA), and FITC-conjugated goat IgG anti-human IgE (from Cappel/ Organon Teknika, Turnout, Belgium). Human myeloma IgE protein was purchased from Bernett Laboratories (Buena Park, CA). Mouse mAb AP2 against platelet GPIIb/IIIa was a gift from Dr. D. Pidard, Paris.

2.2 Flow cytometry

Human platelets were prepared as described in Sect. 2.9 for platelet cytotoxicity, using EDTA (5 % in water) as anticoagulant (1 vol for 9 vol blood) and washing cells with PBS containing 0.01 M EDTA. For IgE receptor detection, 2×10^7 platelets in 100 μl PBS were incubated at

37°C for 30 min with either anti-FcεRI mouse mAb 15-1 and with anti-FcεRII/CD23 mouse mAb 135 for single labeling experiments, or with rabbit polyclonal Ab Rb 55 at 20 µg/ml final concentration in the case of double labeling experiments. In single labeling experiments, the binding of mouse mAb was revealed with FITC-conjugated goat anti-mouse IgG. In double labeling experiments, Rb55 rabbit Ab binding was detected with FITC-conjugated goat anti-rabbit IgG, and 15-1 mouse mAb with PE-conjugated goat anti-mouse IgG1. Unrelated IgG isotype controls were carried out with 20 µg anti-*Toxoplasma* mouse IgG1 mAb or normal rabbit antibody, and control values were subtracted to obtain specific percentage for IgE receptor expression. For IgE binding, platelets were incubated first with either 10 µg human myeloma IgE (when a saturation of IgE receptors was investigated) or with medium (when the labeling of *in vivo*-bound IgE was looked for), followed, after washing, with FITC-conjugated goat IgG anti-human IgE (1/40). The platelet-specific integrin GPIIb/IIIa was occasionally detected with mAb AP2 as a probe of platelet integrity, giving always more than 80 % positive platelets.

2.3 Cell culture

The megakaryocytic cell line DAMI, the eosinophilic cell line EoL3 and the basophilic cell line KU812 were maintained in RPMI 1640 medium supplemented with 10 % FCS and 25 µg/ml gentamycin. Human eosinophils were purified as described [13].

2.4 Northern blot analysis

Total RNA from highly purified platelets was isolated using the guanidine isothiocyanide cesium chloride method [13]. Ten micrograms were fractionated in a 1 % agarose/formaldehyde gel and transferred to nylon membrane (Hybond[®]N, Amersham, Les Ulis, France) in 10 % standard saline citrate (SSC). After ultraviolet cross-linking, the filters were prehybridized for 2 h at 42°C in (40 % formamide, 10 % dextran sulfate, 4 % SSC, 20 mmol/l Tris-HCl pH 7.4, 1 × Denhardt's solution, 20 mg/ml denatured salmon sperm DNA, 0.1 % SDS). Hybridization with the ³²P-labeled probe was performed using the same conditions overnight. After hybridization, the filters were washed 10 min at room temperature in 2 × SSC, 0.05 % SDS and twice in 2 × SSC, 0.05 % SDS at 42°C and 1 × SSC, 0.05 % SDS at 55°C for 30 min each time.

2.5 RT-PCR

Reverse transcription was performed using 2 µg of total RNA in a cDNA synthesis reaction utilizing murine Moloney leukemia virus (MLV) 1 reverse transcriptase as recommended by the supplier (all Gibco, Bethesda Research Laboratories, Gathersburg, MD). PCR was performed by adding 1 µl of the RT product into 50 µl of total volume reaction containing: 1 × buffer (Cetus Corp., Norwalk, CT); 200 mmol of each dNTP (Boehringer Mannheim Biochemicals); 2.5 mM MgCl₂; 20 pmol of each oligonucleotide primer and 0.2 unit of ampli Taq polymerase (Cetus). PCR conditions were 1 min at 94°C,

2 min at 60°C, 3 min at 72°C and 25 cycles for γ subunit and β actin or 35 cycles for β subunit and CD23/FcεRII. The PCR products were electrophoresed on Nusieve/Seakem (3 : 1) agarose gels (FMC Bio Product, Rockland) and visualized by ethidium bromide staining.

2.6 Southern blot analysis

Amplified products were blotted onto Hybond[®]N membranes according to standard conditions. Oligonucleotide probes were labeled with T4 polynucleotide kinase and [³²P]ATP. Hybridization was carried out using the same conditions as described in Sect. 2.4. Oligonucleotide primers and probes were synthesized on a DNA synthesizer (Cyclone plus, Miligen/Biosearch, Burlington, MA) on the basis of the published sequences. All three FcεRII primers were selected in the sequence which is common to a and b forms of CD23: 5' primer (C1): 5'CTGTGGCACTGGGACACCACA3'; 3' primer (C2): 5'TGTGTGCAACACGTGCCCTGAA3'; Internal CD23 primer (C3): 5'TGGACTGGGATTCTGCGCCAT3'. The FcεRI β chain primers were: 5' primer: 5'GGACA-CAGAAAAGTAATAGGAGAG3'; 3' primer: 5'GAT-CAGGATGGTAATTCCCGTT3'. Internal primers were: 5'TTTTCATCATTAAAGCAGGTTATCCATT3'. The FcεRI γ chain primers were: 5' primer: 5'CCAGCAGTG-GTCTTGCTCTTAC3'; 3' primer: 5'GCATGCAGGCA-TATGTGATGCC3'.

2.7 In situ RT-PCR

Cytospin preparations were permeabilized with methanol/acetone (1:1, v/v) for 20 min at –20°C. Cells were fixed in 4 % paraformaldehyde, then washed extensively in diethylpyrocabonate-Tris-buffered saline (DEPC-TBS) and then in water. Air-dried cells were acetylated in 0.1 M triethanolamine buffer for 5 min at 4°C and 0.1 M triethanolamine containing 0.25 % acetic anhydride for 10 min at room temperature. Cells were hybridized with 2.5 ng/µl of antisense oligonucleotides (α1) to human FcεRI α chain gene in 50 % formamide, 10 % dextran sulfate, 300 mM NaCl, 20 mM Tris, pH 7.6, 1 mM EDTA, 1 × Denhardt's solution and 10 mM dithiothreitol (DTT) buffer for 16 h in humidified chamber at 37°C. Cells were then washed in a 50 % formamide, 2 × SSC, 1 mM EDTA, and 10 mM DTT buffer and air dried. First-strand cDNA synthesis was done using MMLV reverse transcriptase (3U/µl) in a buffer of 75 mM KCl, 10 mM Tris, pH 8, 12 mM MgCl₂, 2 µg/µl BSA, 10 mM DTT, containing 1 mM of each dATP, dGTP, dCTP and dTTP. RNase inhibitor (1 µg/µl) was applied to the cells for 3 h in a humidified chamber. Slides were washed extensively in 2 × SSC, water and equilibrated with 1X PCR buffer.

2.8 cDNA amplification

Taq polymerase (5 U/µl; Promega, Madison, WI) was added to a buffer containing 1 mM dATP, dCTP, dGTP, dTTP and 11 digoxigenin-labeled dUTP, 75 mM KCl, 10 mM Tris, pH 8, 10 mM MgCl₂ and 10 pmol/µl of each the 5' (α2) and 3' (α3) oligonucleotides complementary to the human FcεRI α chain gene. The reaction mixture was

added to the slides, and the coverslips were sealed at the edge with nail-polish to prevent desiccation. PCR conditions were 1 min at 94°C, 1 min at 60°C and 1 min at 72°C for 30 cycles. Coverslips were removed, the supernatant aspirated and submitted to tube PCR with primers $\alpha 2$ and $\alpha 3$. The Fc ϵ RI α chain primers were: $\alpha 1$: 5' TACAGTA-ATGTTGAGGGGCTCAG3'; $\alpha 2$: 5'CCTTGACACA-TCCCAGTTCCTCCAACCAT3'; $\alpha 3$: 5'CTGTTCT-TCGCTCCAGATGGCGT3'.

For detection of amplification products, the slides were, after washing in TBS, incubated with 5 % BSA in TBS for 20 min at room temperature and rinsed in TBS. Then the slides were incubated with 1/100 sheep Fab antibody fragments specific for digoxigenin conjugated to alkaline phosphatase or to fluorescein for 60 min at room temperature. Revelation was performed with New Fuchsin kit (Dako, Carpinteria, CA) for alkaline phosphatase and by direct microscopical observation under UV light for FITC.

2.9 Platelet cytotoxicity

Platelets were purified from venous blood (6 vol) of adult healthy volunteers collected on acid citrate-dextrose-C (1 vol) by centrifugation of 5-ml aliquots at 120 g for 15 min at room temperature. The platelet-rich plasma (PRP) was collected in a single volume and centrifuged at 1500 $\times$ g for 15 min after addition of 1 vol ACD-C per 9 vol PRP. Avoiding the recovery of the lowest part of the pellet if contaminated with red cells, the platelets were resuspended in PBS supplemented with 36 mM citric acid, 5 mM glucose, 2 mM CaCl₂, 1 mM MgCl₂, 0.35 % BSA and 100 nM prostaglandin E1 and washed three times in this medium. The last pellet was resuspended in Eagle's Minimum Essential Medium (EMEM) to a cell concentration of 3×10^9 platelets/ml. For cytotoxicity measurement, to 50 μ l platelet suspension were added 50–80 schistosomula in 50 μ l EMEM, 20 μ l serum from allergic patients and unrelated allergen (8 ng grass pollen/ml; Laboratoire des Stallergènes, 92160 Anthony, France) for negative controls or IgE-specific allergen (10 ng *Dermatophagoides pteronyssinus*; Laboratoire des Stallergènes) for positive cytotoxicity, and the indicated concentration of anti-Fc ϵ RI mouse mAb 15-1 or anti-Fc ϵ R2 mouse mAb 135, the final volume being adjusted to 200 μ l of EMEM. As mouse mAb control, anti-*Toxoplasma* mouse mAb was used instead of anti-IgE receptors. The cytotoxicity was evaluated after overnight incubation at 37°C and expressed as the percentage of dead schistosomula.

2.10 F(ab')₂ and Fab fragments of anti-Fc ϵ RI α chain mAb

mAb 15-1 (mouse IgG1) against the α chain of Fc ϵ RI was digested with ficin [18] to produce F(ab')₂ fragments, which were reduced by 2-ME to give Fab fragments. Control gel electrophoresis of IgG and fragments confirmed the efficiency of the digestion.

3 Results

3.1 Fc ϵ RI is expressed by platelets

The surface expression of Fc ϵ RI α chain and Fc ϵ R2/CD23 on human platelets was investigated by flow cytometry using the 15-1 mouse mAb specific for the extracytoplasmic α chain of Fc ϵ RI containing the IgE-binding

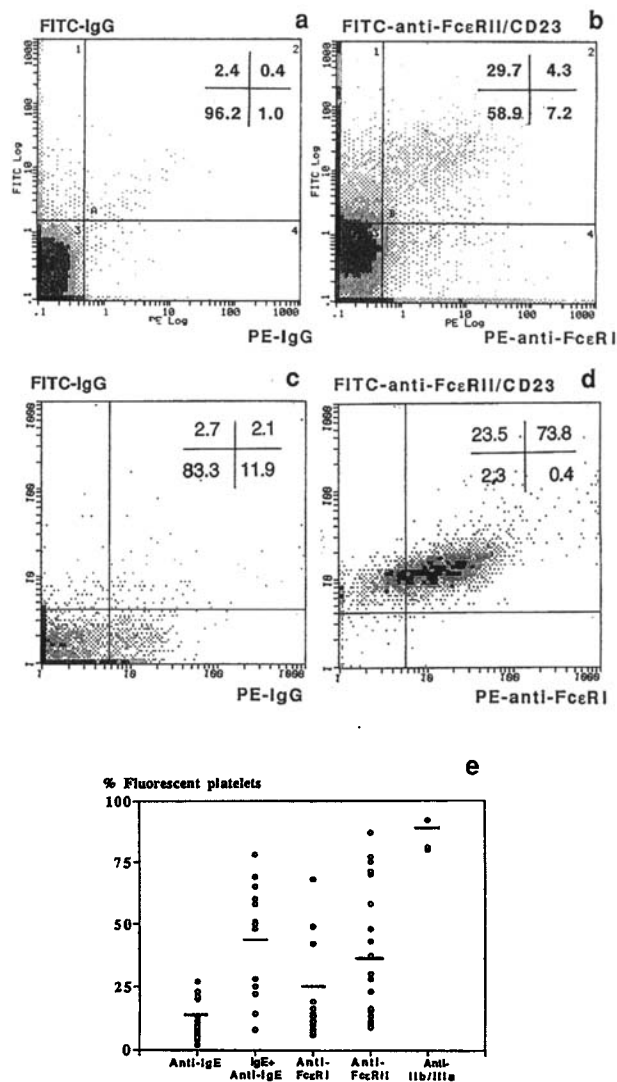


Figure 1. Flow cytometric analysis of human blood platelets and DAMI cells. A representative double-labeling experiment of platelets, performed with mouse mAb 15.1 for Fc ϵ RI and rabbit polyclonal antiserum Rb55 for Fc ϵ R2/CD23 and detected with PE-conjugated goat anti-mouse IgG1 or FITC-conjugated goat anti-rabbit IgG, respectively, is illustrated in (b), with (a) as isotype control. The mean of nine different donors was 22.5 ± 12.0 for Fc ϵ R2/CD23 alone, 8.9 ± 10.1 for double labeling, and 8.6 ± 4.6 for Fc ϵ RI alone. The labeling of the megakaryocytic cell line DAMI is illustrated in (c) (isotypic controls) and (d) (anti-IgE receptors). (e) shows the percent of positive cells obtained with platelets from various donors, labeled with mouse mAb 15.1 for Fc ϵ RI, and with mouse mAb 135 for Fc ϵ R2/CD23, both revealed with FITC-conjugated goat anti-mouse IgG. IgE-positive cells were revealed with FITC-conjugated goat IgG anti-human IgE, and GPIIb/IIIa integrin with mouse mAb AP2 and FITC-conjugated goat anti-mouse IgG.

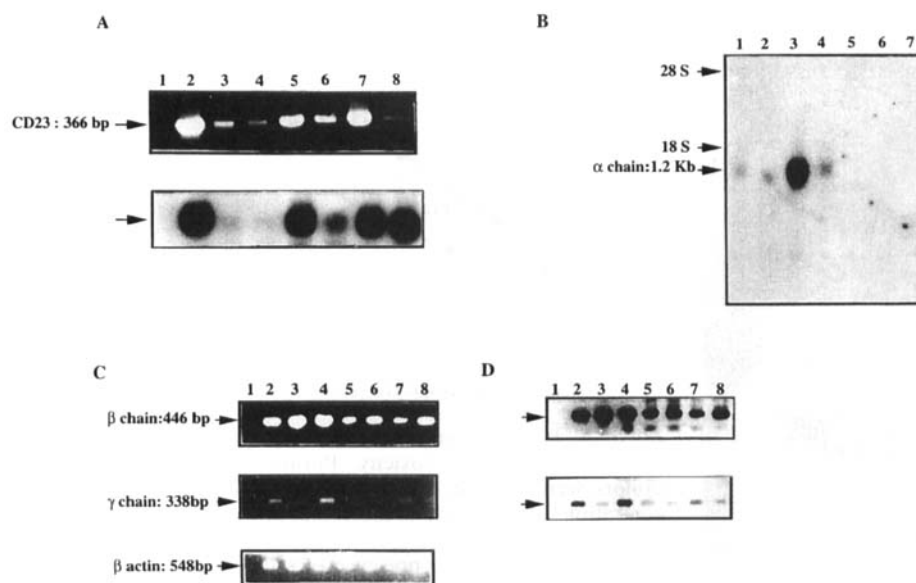


Figure 2. Molecular characterization of FcεRI and FcεRII/CD23 in human platelets. (A) RT-PCR analysis of FcεRII/CD23. Lane 1: without cDNA; lane 2: positive control eosinophilic cell line EoL3; lane 3: purified eosinophils from a hypereosinophilic patient; lane 4: human megakaryocyte cell line DAMI; lanes 5–8: purified platelets from four different donors. CD23-specific primers (C1 and C2) were used in the PCR step (35 cycles). The Southern blot was performed with internal specific primer C3. (B) Northern blot analysis of α chain transcripts of high-affinity receptor FcεRI. Each line represents 10 μg of total RNA. The probe used was the BamHX-XhoI fragment corresponding to human α chain cDNA of FcεRI. Lane 1: KU812 human basophilic cell line; lane 2: purified eosinophils from a hypereosinophilic patient; lane 3: human megakaryocyte cell line DAMI; lanes 4–7: purified platelets from the same donors as in Fig. 2A. (C) RT-PCR analysis of FcεRI β, γ subunits of FcεRI and β actin used as control. Lane 1: negative control: no cDNA was added before the amplification step; lane 2: purified eosinophil from a hypereosinophilic patient; lane 3: KU812 human basophilic cell line; lane 3: human megakaryocytic cell line DAMI; lanes 5–8: purified platelets from four different donors. Twenty-five cycles were performed for β actin and FcεRI γ chain and 35 in the case of FcεRI β chain. (D) Southern blot analysis corresponding to the β and γ subunits; each lane was as described in (C).

domain, and the 135 mouse mAb prepared against the IgE-binding domain of B lymphocyte CD23. Anti-FcεRI and anti-FcεRII mAb were able to bind to purified platelets from normal blood donors with a respective mean of 22 % positive platelets for anti-FcεRI mAb and 34 % for anti-FcεRII/CD23 mAb (Fig. 1e). A large heterogeneity of the expression of both IgE receptors among individual subjects was shown by this technique, similarly to the heterogeneous expression of myeloma IgE binding. Cells of the megakaryocytic DAMI cell line expressed FcεRI (60 % of cells), and FcεRII/CD23 (92 %). The next question was the level of co-expression of these receptors on the same individual cells. Based on nine individual donors, double-labeling experiments (Fig. 1a and b for one donor) showed that only 8.9 % platelets co-expressed FcεRI and FcεRII/CD23. A similar proportion expressed only FcεRI (8.6 %), whereas FcεRII/CD23-positive/FcεRI-negative platelets represented 22.5 % of the cell population and 60.0 % were negative for both IgE receptors. In the case of DAMI cells, all FcεRI-positive cells were simultaneously FcεRII/CD23-positive (Fig. 1c and d).

3.2 Presence of mRNA for FcεRI and FcεRII/CD23 in platelets and megakaryocytes

The expression of FcεRII/CD23 has been shown in human platelets [8]. However, there has been no definitive proof of FcεRII/CD23 synthesis by platelets. To investigate the presence of FcεRII/CD23 mRNA in human platelets, RT-PCR was performed on RNA prepared from highly purified

platelets or from the megakaryocytic cell line DAMI. As shown in Fig. 2A, a specific band corresponding to the predicted size of CD23 and confirmed by Southern blot was obtained with platelet mRNA preparations from four different donors (lane 5–8), or from the DAMI cell line (lane 4). Human eosinophils (lane 3) and the EoL3 cell line (lane 2) were used as positive controls. Northern hybridization was then performed to detect the presence of mRNA encoding the FcεRI α chain in the same platelet mRNA preparations. As shown in Fig. 2B, a signal at 1.2 kb was detected in the DAMI cells (lane 3), in one platelet preparation (lane 4), in eosinophils (lane 2) and in the basophilic cell line KU812 (95 % positive for FcεRI in FACS analysis [13]), used as a positive control (lane 1). The question remained whether platelets could also express the β and γ chains of FcεRI. Due to the limited amount of RNA from purified platelets, a more sensitive Northern analysis with poly-A⁺ RNA could not be carried out. We therefore used RT-PCR to detect β and γ messengers. The amplified material obtained from platelets, DAMI, KU812 or eosinophils revealed a band of the predicted size for β and γ chain of FcεRI in all tested cells (Fig. 2C). The specificity of the fragments was confirmed by restriction enzymes (data not shown) and Southern blotting with internal oligonucleotides specific for each subunits (Fig. 2D).

To ascertain that platelets and their precursors could express the FcεRI α chain, *in situ* RT-PCR was performed on cytocentrifuged preparations of platelets or DAMI megakaryocytic cells. As shown in Fig. 3, which illustrates

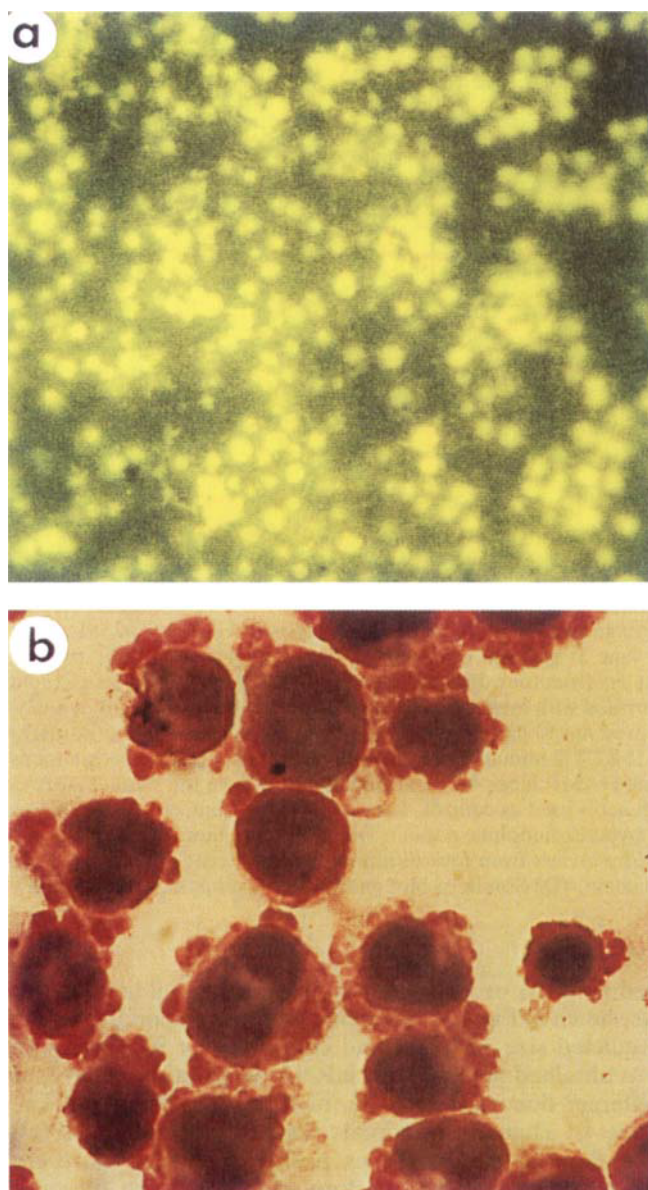


Figure 3. Detection of FcεRI α chain messenger in human platelets and megakaryocytic cell line (DAMI) by *in situ* RT-PCR. (a) Human purified platelets from one normal donor show a positive staining when FITC-conjugated anti-digoxigenin antibody was used in the revelation step. (b) DAMI cell line positively stained for amplified α chain cDNA products after incubation with anti-digoxigenin antibody conjugated to alkaline phosphatase and New Fuchsin kit (red color). Cells were counterstained with hematoxylin.

a positive staining of amplified products with digoxigenin-labeled nucleotides and anti-digoxigenin fluorescein-labeled Ab or alkaline phosphatase conjugates, the majority of platelets (Fig. 3a) and of DAMI cells (Fig. 3b) expressed FcεRI α chain mRNA. The absence of signal when reverse transcriptase was omitted showed that synthesized cDNA rather than genomic DNA was detected by this *in situ* RT-PCR. No signal was detected in the absence of Taq polymerase.

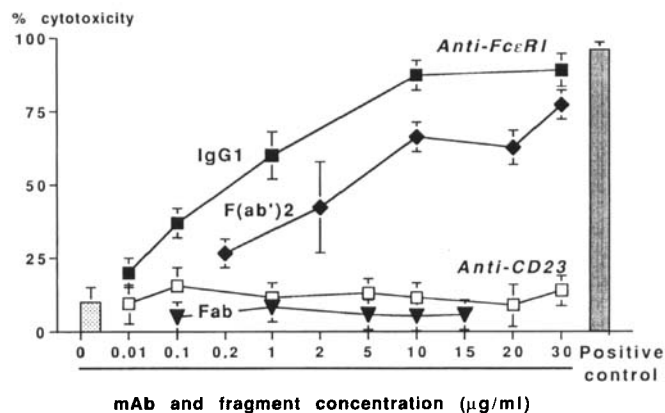


Figure 4. Effects of anti-FcεR antibodies and their fragments on platelet-mediated cytotoxicity. Purified platelets from normal donors were incubated with normal serum and increasing concentrations of either whole molecule (■), F(ab')₂ (◆), Fab (▼) of anti-FcεRI mouse mAb 15-1, or anti-FcεRII/CD23 (□) mAb 135 whole molecule, in the presence of schistosome larvae. Positive control was obtained with IgE-rich serum from a patient with *Dermatophagoides pteronyssinus*-induced asthma and specific allergen (■), whereas negative control was obtained with serum from a normal donor (□). The cytotoxicity was evaluated after overnight incubation at 37°C and expressed as the percentage of dead schistosomula (mean ± SD of five experiments in triplicate).

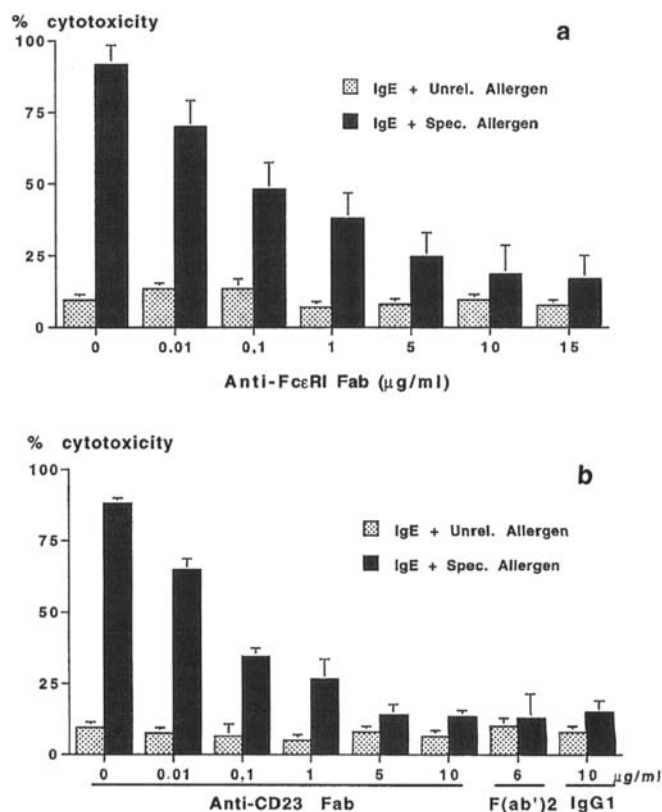


Figure 5. Inhibitory effect of Fab fragments of anti-FcεRI and anti-FcεRII/CD23 mAb on IgE-mediated cytotoxicity of human blood platelets. Purified platelets were incubated with IgE-rich serum from a mite-sensitive asthmatic and unrelated allergen (grass pollens □), or specific allergen (*Dermatophagoides pteronyssinus* ■), and increasing concentrations of Fab fragments of anti-FcεRI mAb 15-1 (a) or Fab fragments of anti-FcεRII/CD23 mAb 135 (b). The cytotoxicity was evaluated as in Fig. 4 (mean ± SD of three experiments in triplicate).

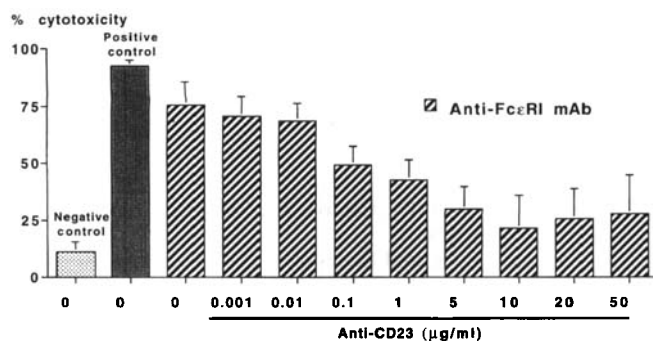


Figure 6. Inhibition of FcεRI-induced cytotoxicity of platelets by anti-FcεRII/CD23 mAb. Purified platelets were incubated with serum from normal donors (□ background activity) or with the IgE-rich serum of a patient with house dust-induced asthma and mite allergen (■ positive control), or with anti-FcεRI antibody at a concentration (10 μg/ml) mediating an optimal stimulation of platelets (▨). Increasing concentrations of anti-FcεRII mAb 135 added 30 min before mAb 15-1 induced a dose-dependent inhibition of the cytotoxicity.

3.3 FcεRI triggers cytotoxic functions of platelet

Previous studies had shown that platelets could be activated in a specific manner by incubation with IgE antibodies followed by the addition of specific allergen or by cross-linking with anti-IgE antibody [8]. As a consequence of this activation process, platelets released cytotoxic mediators, including oxygen metabolites or free radicals, which, in the present study, were evaluated by the killing of schistosome larvae. As shown in Fig. 4, the cross-linking of FcεRI also induced platelet activation: isolated human blood platelets expressed dose-dependent cytotoxic properties towards schistosome larvae when incubated with mAb 15-1, or with its F(ab')₂ fragments, reaching a plateau with 10 μg antibody/ml, whereas Fab fragments were inactive in this process. In contrast, cross-linking of FcεRII/CD23, which is presented here for mAb 135 whole molecules (Fig. 4) but has also been observed with different polyclonal and monoclonal antibodies to CD23 [9], was unable to induce platelet cytotoxicity. Fab fragments of anti-FcεRI α chain, which did not lead to receptor cross-linking, were not only unable to trigger platelet cytotoxicity but produced a dose-dependent inhibition of the IgE-mediated stimulation of platelets by allergen-specific IgE (Fig. 5a). Antibodies to CD23, either bivalent, *i.e.* IgG1 and F(ab')₂ or monovalent, *i.e.* Fab fragments, which did not induce cytotoxicity, were able to inhibit the IgE-dependent stimulation of platelets (Fig. 5b).

3.4 Interactions between FcεRII/CD23 and FcεRI functions

We next addressed the question of the respective role of FcεRI and FcεRII/CD23 in platelets, which was investigated by adding simultaneously both anti-FcεRI and anti-FcεRII antibodies in the cytotoxicity assay. When an optimal amount of anti-FcεRI mAb was used to induce cytotoxicity (*i.e.* 10 μg/ml as shown in Fig. 4), increasing concentrations of anti-FcεRII/CD23 mAb inhibited the cytotoxic process in a dose-dependent manner (Fig. 6), suggesting a down-regulation of FcεRII/CD23 on FcεRI. As already shown in Fig. 5b, a similar dose-dependent inhibition of platelet

cytotoxicity was observed with anti-FcεRII/CD23 mAb when allergen-specific IgE was used as triggering agent.

4 Discussion

Whereas platelets have been clearly involved in IgE-dependent release of cytotoxic mediators *in vitro* as well as in immune protection to schistosome infection *in vivo* [1–4, 19, 20], the precise underlying molecular mechanisms have not been elucidated yet. A role for FcεRII/CD23 was first proposed, based mainly on the ability of anti-FcεRII/CD23 antibodies to inhibit IgE binding to platelets and the expression of IgE-dependent cytotoxic properties by these cells. However, some discrepancies between CD23 expression and IgE-binding which were evidenced, among other things by a bimodal curve in Scatchard analysis of IgE binding [8], together with the recent demonstration that FcεRI expressed by eosinophils also participated in anti-parasite cytotoxicity [13], led us to investigate FcεRI expression and function in platelets.

The presence of FcεRI α-chains on human platelets was demonstrated here by flow cytometry using mAb 15-1 directed against the FcεRI α chain. Even if some heterogeneity appeared among donors for both structures involved in IgE binding, a significant correlation was observed between the level of IgE binding and that of anti-FcεRI α-chain antibody, similarly to the situation described for eosinophils [13]. Northern blot analysis and PCR amplification of platelet RNA, either after RNA extraction or *in situ*, have allowed the demonstration that these anucleated blood elements have the capacity to synthesize all three chains of FcεRI, similarly to eosinophils, but in contrast with monocytes which do not express the β chains of FcεRI [14]. Only one platelet extract out of four expressed sufficient mRNA for the α-chain of FcεRI to be revealed without amplification. This is neither incompatible with the existence of low levels of the corresponding mRNA, as shown by *in situ* RT-PCR, nor with the surface expression of the α-chain protein by platelets which are short-living end-products of megakaryocytes. The ability of platelets to express FcεRI was confirmed by its significant presence on the human megakaryocytic cell line DAMI. Its identification on normal bone marrow megakaryocytes is presently under investigation. On the other hand, platelets as well as human megakaryocytic cell lines can also express FcεRII/CD23 mRNA, an observation which has not been reported previously. Taken together, these results show the presence of FcεRI as well as FcεRII/CD23 in human platelets and the megakaryocytic cell lineage.

Platelet FcεRI apparently represents one membrane structure carrying IgE-dependent functions expressed by platelet cytotoxicity, since its cross-linking by anti-receptor mAb reproduced the stimulation process mediated by anti-parasite or allergen-specific IgE antibodies. Similarly, the inhibition of their IgE-dependent killing ability by anti-FcεRI mAb gives this receptor a crucial role in the generation of platelet effector functions. The involvement of platelet FcεRI has been postulated recently, together with that of FcεRII/CD23, in the development of T cell contact sensitivity through the local release of serotonin by platelets [21]. The participation of FcεRII/CD23 in the IgE-mediated cytotoxicity of blood platelets, which was ini-

tially considered as primary if not exclusive, appears now in a new light. We confirm here the previously reported inhibitory effect of anti-FcεRII/CD23 antibodies on the IgE-dependent stimulation of platelets [8]. The inhibition of IgE synthesis in the rat after anti-FcεRII/CD23 antibody or Fab treatment has also been reported [22]. But the yet unexplained inability of these anti-FcεRII/CD23 antibodies to induce by themselves the generation of cytotoxic mediators in platelets or in mononuclear phagocytes, the putative normal consequence of a cross-linking of adjacent receptors, finds here the beginning of an explanation. According to the key observation presented here, FcεRII/CD23 seems to express regulatory properties: in the presence of IgE, which has been shown to stabilize CD23 [23], a full expression of FcεRI functions is favored. The addition of anti-FcεRII/CD23 antibody, by inhibiting the binding of IgE [8], could induce a destabilization of CD23, accelerating its autocatalytic cleavage or its internalization, leading to the observed inhibitory effect. A pathway for this CD23-mediated inhibition could be associated with CD23-linked trans-activation signals, which could be a prerequisite for a full expression of FcεRI. In this perspective, FcεRI functions would be abolished as soon as CD23 has been catalyzed or endocytosed, or if normal IgE-CD23 interaction is impaired by specific antibody. Recently, it has been described in mast cells that the capacity of FcεRI to trigger mediator release is inhibited by its cross-linking to FcγRII, the low-affinity IgG receptor [24], through immunoreceptor tyrosine-based inhibition motifs [25]. Whether the control of CD23 on FcεRI functions was expressed through intracellular signals mediated by a similar interaction with immunoreceptor tyrosine-based activation motif domains on β and γ chains of the high affinity receptor for IgE, or through extracellular soluble mediators, is open to further investigations. The apparent separation between FcεRII/CD23-positive and FcεRI-positive platelets is in favor of the second hypothesis.

If CD23 controls the functions of platelet FcεRI, these results open interesting perspectives not only in this particular cell type but on other cell populations where both receptors have been reported, including mast cells. The problem is now to define the kinetics of expression of these receptors by cells and a possible link with cell differentiation and maturation stage. Recent observations on murine B lymphocytes have shown that the gene for FcεRII/CD23 is actively transcribed during all stages of the cell development, but that the surface expression of the protein structure is not correlated with the mRNA content and is regulated through post-transcriptional mechanisms [26]. In the same perspective of IgE receptor expression on blood platelets, we have reported an inducing role for lymphocytes through IFN-γ [27]. Finally, the metabolic link between both IgE-binding structures on platelet membrane, and possibly other cells, is of importance in the physiological and pathophysiological functions of IgE-positive cells. Their balance could explain the on and off activity of cells involved in immediate hypersensitivity reactions as observed during specific immunotherapy in patients with systemic reactivity to hymenoptera venom [28]. Therefore, in the context of the cell network involved in allergic diseases, a better understanding of the mechanisms concerned might be of consequence in the elaboration of therapeutic measures in the treatment of allergic disorders.

We are very much indebted to Mrs. Brigitte Quatannens for her invaluable help with FACS analysis. A.S.G. was granted by the Fondation Mérieux.

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