

Expression of a Functional FcεRI on Rat Eosinophils and Macrophages

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Besides its crucial role in type I hypersensitivity reactions, IgE is involved in anti-parasite immunity. This role has been clearly demonstrated in both human and rat schistosomiasis, but remains controversial in the mouse. Since the cellular distribution of the high affinity IgE receptor, FcεRI, differs in humans and mice, it might explain the differences in effector function of IgE between the two species. In humans, eosinophils and macrophages induce IgE-dependent cytotoxicity toward *Schistosoma mansoni* larvae, which involves FcεRI in the case of eosinophils. In the present study, we have investigated the expression and function of FcεRI in rat eosinophils and macrophages. We demonstrate, by flow cytometry, fluorescence microscopy, and Western blot analysis, that in rats, as in humans, a functional αγ₂ trimeric FcεRI is expressed on eosinophils and macrophages. We also show that these two cell types can induce IgE-mediated, FcεRI-dependent cellular cytotoxicity toward schistosomula. These results thus provide a molecular basis for the differences observed between rat and mouse regarding IgE-mediated anti-parasite immunity. *The Journal of Immunology*, 2000, 165: 1266–1271.

It is well documented that IgE and FcεRI are key players in allergic reactions. Receptor-bearing mast cells and basophils are able to release, upon engagement with IgE and multivalent Ag, inflammatory mediators (histamine, leukotrienes) as well as proinflammatory and immunoregulatory cytokines (1). Besides its expression on mast cells and basophils as an αβγ₂ tetramer, FcεRI is also expressed on human Langerhans cells (2, 3), monocytes (4), eosinophils (5), and platelets (6). This extended cellular distribution allows IgE and FcεRI to be involved in allergen presentation (7) as well as in in vitro cytotoxicity reactions (Ab-dependent cellular cytotoxicity; ADCC)² toward parasite targets such as larvae from the trematode parasite *Schistosoma mansoni* (5, 6). However, it has been recently demonstrated that murine eosinophils and macrophages did not express FcεRI (8, 9), whereas this receptor could be detected on the same cell types in transgenic mice expressing the human FcεRIα under the control of its own promoter elements (9, 10).

In rats and humans, resistance against schistosome infections involves, among others, IgE-dependent mechanisms (11). Indeed, several immunoepidemiological studies have evidenced a negative correlation between IgE levels and rates of reinfection by the three species of *Schistosoma* that are pathogen for humans: namely *S. mansoni* (12, 13), *S. haematobium* (14), and *S. japonicum* (15). Besides such indirect evidences, a more direct demonstration of the role of IgE in protective immunity was brought both in vitro and in vivo. In humans and rats, IgE was shown to induce ADCC

reactions toward schistosome larvae in the presence of eosinophils, monocytes/macrophages, or platelets (11). Furthermore, immunization of rats according to protocols leading to IgE production (16, 17), passive transfer of IgE rich-serum from *S. mansoni*-infected rats or of anti-*S. mansoni* rat IgE mAb to naive recipient rats (18) led to a significant level of protection to a challenge infection. Such a protective effect was also observed when platelets (19), eosinophils, or macrophages (20), obtained from infected animals and bearing cytophilic IgE, were transferred to naive rats. In mice, various studies about the protective role of IgE in schistosomiasis have led to divergent conclusions (21–25). IgE and eosinophils have also been associated with resistance against other helminthic parasites, such as *Trichinella* (26) and *Necator* (27).

While expression of functional IgE receptors has been demonstrated on rat macrophages and eosinophils (28–31), their molecular nature has not been characterized. Due to the similarities of IgE effector functions in humans and rats in vivo and in vitro and to the contradictory results from the various studies on murine models, we hypothesized that the discrepancies between mice and rats regarding the involvement of IgE in anti-schistosome immunity might be due to differences in the cellular distribution of FcεRI.

In the present work we have investigated the cellular distribution and function of FcεRI in rat eosinophils and macrophages. Our results provide the first explanation for the long-lasting controversy about the rat vs the mouse as an animal model for parasitic infections.

Materials and Methods

Abs and reagents

Anti-rat FcεRIα Abs were gifts from Dr. R. P. Siraganian (National Institutes of Health, Bethesda, MD; BC4) (32) and Dr. T. J. Flemming and J.-P. Kinet (Harvard, Boston, MA; 3A92). Both Abs are mouse IgG1. Mouse monoclonal anti-rat FcRβ (JRK) (33), which also recognizes mouse FcRβ, and the anti-FcRγ rabbit antiserum (no. 934) recognizing human, murine, and rat molecules (34) were also provided by Dr. J.-P. Kinet. Anti-human CD23 antiserum (Rb 103A) recognizing rat CD23 (35) was provided by Dr. J.-Y. Bonnefoy (Geneva, Switzerland). IgE from rat myeloma (IR 162), biotinylated IR 162, and anti-rat IgE (MARE-1) were obtained from LO-Immex (Louvain, Belgium).

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² Abbreviations used in this paper: ADCC, Ab-dependent cellular cytotoxicity; St-PE, PE-conjugated streptavidin; RBL, rat basophil leukemia.

S. mansoni cycle

A Puerto Rican strain of *S. mansoni* was maintained in *Biomphalaria glabrata* snail as the invertebrate intermediate host and in the golden hamster, *Mesocricetus auratus*, as the vertebrate definitive host. Skin schistosomula for cytotoxicity (ADCC) experiments were collected in MEM after application of cercariae to isolated pieces of Swiss mouse abdominal skin for 3 h.

Animals and treatments

Lou rats (8–16 wk old) were kept and bred in the facility of the Institut Pasteur de Lille. Unless specified otherwise, animals were i.p. injected with 5 ml of thioglycolate (5%). Six-month-old animals were used for peritoneal mast cell purification.

Cells

Rat basophil leukemia (RBL-2H3) cells were kept in complete RPMI medium supplemented with 10% FCS.

Rat peritoneal cells were obtained, 4 days after thioglycolate injection by lavage of the peritoneal cavity with PBS and were directly analyzed by flow cytometry or used for eosinophil and macrophage purification.

Eosinophils (60–80% purity estimated after May-Grünwald staining) obtained by removal of adherent cells (mainly macrophages) after overnight culture in complete RPMI medium with 10 ng/ml recombinant human IL-5 were used for ADCC experiments. For receptor up-regulation experiments, cells were maintained for 4 days in complete RPMI medium supplemented with 10 ng/ml human IL-5 in the presence or the absence of 5 μ g/ml rat IgE, then analyzed by flow cytometry.

Macrophages were obtained as adherent cells after 30-min plating and were used in ADCC experiments. Purity was typically 90–95%.

Mast cells from 6-mo-old naive rats were purified on a 22% metrizamide gradient (36). Purity estimated on cytospin preparations after May-Grünwald staining was 97%.

Flow cytometry

Unless specified otherwise, all incubations for staining were performed on $2-5 \times 10^5$ cells in 100 μ l with 10 μ g/ml Ab at 4°C for 30 min in PBS containing 0.1% BSA and 0.05% sodium azide. Eosinophils and macrophages were identified on the basis of their forward and side scatter. Fc ϵ RI expression was analyzed by binding of either unlabeled rat IgE revealed with biotinylated mouse anti-rat IgE and PE-conjugated streptavidin (St-PE; 5 μ g/ml) or with biotinylated rat IgE and St-PE after incubation with 150 μ g/ml nonspecific rat IgG (to block potential binding to Fc γ R) and anti-CD23 antiserum (1/100 dilution; to prevent IgE binding to this receptor). Binding specificity was verified by competition with 150 μ g/ml unlabeled anti-rat Fc ϵ RI α .

Detection of Fc ϵ R β and Fc ϵ R γ was performed by intracellular staining of peritoneal cells. Cells were fixed for 10 min at room temperature with 2% freshly prepared paraformaldehyde, washed twice with PBS, then permeabilized for 10 min at room temperature with 0.5% saponin in PBS containing 1% BSA. The subsequent incubation and washing steps were performed in the same solution. Anti-Fc ϵ R β (1/100 dilution) was revealed using a PE-conjugated donkey F(ab')₂ anti-mouse IgG (4 μ g/ml) and anti-Fc ϵ R γ (1/400 dilution) using an FITC-conjugated goat F(ab')₂ anti-rabbit IgG (7.5 μ g/ml). A final wash in PBS was performed before analysis.

Immunofluorescence

Purified populations of Fc ϵ RI-positive eosinophils and macrophages were obtained by sorting peritoneal cells according to their forward and side scatter and their positivity for Fc ϵ RI, using an ELITE cell sorter (Coulter, Hialeah, FL), after surface staining performed as described above. The purity of sorted cells was assessed on cytospin preparations after staining with May-Grünwald (RAL, Martillac, France). Purity was 100% for eosinophils and 98% for macrophages. Sorted cells were fixed with 2% paraformaldehyde, then washed with PBS before mounting. Preparations were observed with a $\times 63$ Plan-Apochromat objective on an Axiophot 2 microscope (Zeiss, New York, NY) equipped with a digital camera.

Western blot

Subunit composition of the receptor on eosinophils and macrophages was analyzed by Western blot on purified populations obtained by sorting of unlabeled peritoneal cells from thioglycolate-injected rats. Sorted eosinophils and macrophages as well as control purified peritoneal mast cells from naive 6-mo-old animals and RBL cells were resuspended in PBS containing 0.1% BSA and 0.5% sodium azide at a concentration of 10^7 cells/ml and incubated with 150 μ g/ml nonspecific rat IgG and a 1/100 dilution of anti-CD23 rabbit antiserum. Cells were incubated with 10

μ g/ml biotinylated rat IgE, washed with PBS, then lysed in 1% digitonin (Gallager and Schlessinger Carle Place, NY) as previously described (9, 37). As a preclearing step, lysates were first incubated with agarose beads for 1 h. Surface-expressed receptors were then immunoprecipitated with avidin-agarose beads. Pooled material (9.3×10^6 eosinophils, 8.6×10^6 macrophages) obtained from three sorting experiments (12 rats) as well as 5×10^6 RBL and 3.6×10^6 purified peritoneal mast cells were loaded on a 14% reducing SDS-PAGE (for detection of Fc ϵ R β and Fc ϵ R γ). After transfer on polyvinylidene difluoride membrane, samples were probed with anti-Fc ϵ R β (1/2500 dilution) and anti-Fc ϵ R γ (1/5000 dilution) Abs. HRP-conjugated secondary Abs were revealed by chemiluminescence using SuperSignal (Pierce, Rockford, IL) according to the manufacturer's protocol.

Fc ϵ RI α was detected in lysates from 5×10^6 eosinophils, 6.5×10^6 macrophages, 5×10^6 RBL, and from 3.6×10^6 purified peritoneal mast cells (used as a control) after sequential immunoprecipitation with normal rabbit serum bound to protein A-Sepharose (preclearing) and with anti-Fc ϵ R γ serum bound to protein A-Sepharose. Immunoprecipitated material was loaded on an 8% nonreducing gel, transferred on polyvinylidene difluoride, then probed with anti-Fc ϵ RI α (BC4; 1/2500 dilution).

Ab-dependent cellular cytotoxicity

Effector cells (4×10^5 ; eosinophils or macrophages) were incubated in flat-bottom 96-well plates for 5 h with a 1/20 dilution from serum from 63-day-infected rats (containing 5 μ g/ml IgE) or with serum from uninfected animals (as a control) in 100 μ l of complete RPMI medium. Schistosomula (100 larvae) were then added to the effector cells in a final volume of 200 μ l. Some cell samples were preincubated with 150 μ g/ml anti-Fc ϵ RI α or with normal mouse IgG before the addition of rat serum. Additional controls were performed by incubating the cells with anti-Fc ϵ RI in the absence of serum or with infected rat serum depleted in IgE by overnight incubation at 4°C with 30 μ g/ml anti-rat IgE adsorbed to protein A-Sepharose beads. Cytotoxicity was estimated 48 h later and was expressed as the percentage of dead schistosomula evaluated microscopically. Experiments were performed in duplicate.

Results

Expression of Fc ϵ RI by rat eosinophils and macrophages

The expression of Fc ϵ RI by peritoneal eosinophils or macrophages from normal rats was investigated by flow cytometry using rat myeloma IgE. The experiments were performed after saturation of Fc γ Rs, IgG receptors, and Fc ϵ RII/CD23, the low affinity IgE receptor, with an excess of unlabeled IgG and anti-CD23 Ab, respectively. Under these conditions no IgE binding could be detected on any cell type (data not shown), suggesting that Fc ϵ RI was not expressed or was undetectable on these resting cells.

We next analyzed peritoneal cells 4 days after the injection of thioglycolate. As in the mouse, eosinophils and macrophages were easily and accurately identified on the basis of their forward and side scatter parameters. Eosinophils (15–20% of the total peritoneal cells) were small and displayed the highest granularity (38), whereas macrophages (70–80%) were the largest cells in the peritoneum (39) (Fig. 1a). Mast cells were virtually absent from the peritoneum from these animals (1% or less of the peritoneal population). A small eosinophil subpopulation (~5%) exhibited strong IgE binding, which was completely inhibited by preincubation of cells with anti-Fc ϵ RI α mAb (Fig. 1b). Two different anti-Fc ϵ RI α mAb gave similar results. These findings indicate that rat eosinophils are able to express Fc ϵ RI. Likewise, a higher proportion (20–30%) of macrophages could bind IgE, and this binding was very significantly inhibited by preincubation with anti-Fc ϵ RI α Abs (Fig. 1c). However, the mean fluorescence intensity, reflecting the receptor number, was about 4-fold lower than that for eosinophils.

Fc ϵ RI-positive eosinophils and macrophages were then sorted according to both scatter and fluorescence parameters and were examined by microscopy. Purified populations (98–100% purity) were very homogeneous in size and aspect. After May-Grünwald staining, eosinophils appeared small, with a highly granular and eosinophilic cytoplasm and a doughnut- or eight-shaped nucleus

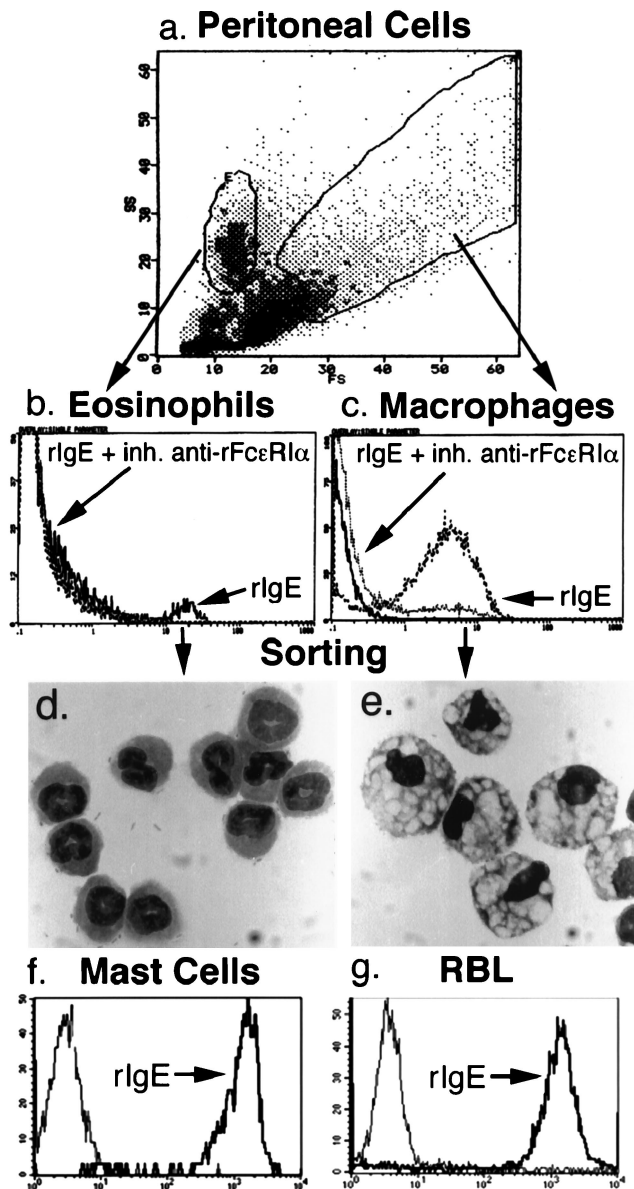


FIGURE 1. Surface expression of FcεRI on eosinophils and macrophages. *a–c*, Flow cytometric analysis of peritoneal cells 4 days after thioglycolate injection. *a*, Scatter representation. *b* and *c*, FcεRI expression on gated eosinophils (*b*) and macrophages (*c*). Binding of rat IgE is detected with biotinylated anti-rat IgE and St-PE in the presence or the absence of preincubation with anti-rat FcεRIα. *d* and *e*, May-Grünwald staining of cytopsin preparations of sorted FcεRI-positive eosinophils (*d*) and macrophages (*e*). *f* and *g*, Flow cytometric analysis of purified peritoneal mast cells from naive rats (*f*) and RBL cells (*g*).

(Fig. 1*d*). Macrophages were much larger, with prominent cytoplasmic vacuoles (Fig. 1*e*). No contaminating granulated mast cells could be detected on either preparation.

Purified peritoneal mast cells from 6-mo-old naive animals (Fig. 1*f*) and RBL cells (Fig. 1*g*), used as a control, displayed much higher FcεRI expression when stained using the same experimental procedure.

FcεRI-positive eosinophils and macrophages were analyzed by immunofluorescence microscopy (Fig. 2). As observed on peritoneal mast cells and RBL cells, a typical speckled pattern of fluorescence was detected on both sorted eosinophils and macrophages. As expected, expression levels in these two populations were lower than that in the cell line.

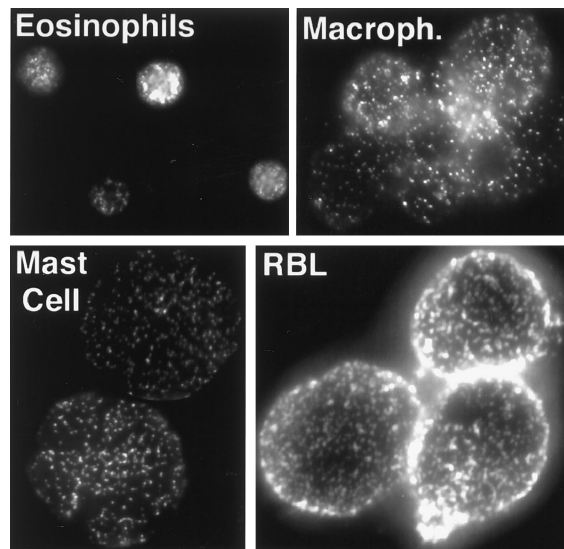


FIGURE 2. Immunofluorescence microscopy analysis FcεRI-positive cells. Sorted eosinophils and macrophages, purified peritoneal mast cells, and RBL cells were stained as described in Fig. 1 and were visualized by fluorescence microscopy.

FcεRI molecular structure

To determine whether FcεRI was expressed as a trimeric $\alpha\gamma_2$ or a tetrameric $\alpha\beta\gamma_2$ structure on eosinophils and macrophages, the presence of FcRβ and FcRγ was examined by flow cytometry after cell permeabilization with saponin, using specific anti-FcRβ and anti-FcRγ Ab (Fig. 3). FcRβ was detected in neither eosinophils nor macrophages (Fig. 3, *a* and *c*), in contrast to peritoneal mast cells and RBL cells (Fig. 3, *e* and *g*). This further confirms that the gated eosinophil and macrophage populations did not contain mast cells, which do express an $\alpha\beta\gamma_2$ receptor. By contrast, FcRγ, which also associates with FcγRI and FcγRIII, was virtually detected in 100% eosinophils and macrophages (Fig. 3, *b* and *d*). As expected, peritoneal mast cells and RBL cells were positive for both FcRβ and FcRγ (Fig. 3, *e–h*).

To confirm that the receptor expressed by eosinophils and macrophages was lacking FcRβ, we analyzed the structure of surface-expressed receptors by Western blot on purified populations. Purified eosinophils and macrophages as well as peritoneal mast cells and RBL cells were incubated with biotinylated rat IgE, as described for flow cytometry. After lysis with digitonin, a very mild detergent known to preserve noncovalent associations between the receptor subunits (9), immunoprecipitation was performed using avidin-agarose beads. After SDS-PAGE, the membrane was probed with anti-FcRβ and anti-FcRγ Abs. As observed by flow cytometry after permeabilization, FcRβ was detected on neither eosinophils nor macrophages, while a strong band corresponding to FcRβ was detected in the lysate from RBL and mast cells (Fig. 4, *middle part*). On the other hand, two bands, probably corresponding to unphosphorylated and phosphorylated forms of FcRγ (40), were detected on both eosinophils and macrophages (Fig. 4, *lower part*). Due to the higher receptor expression in peritoneal mast cells and RBL and to the increased detergent stability of the tetrameric receptor compared with the trimeric structure (9), the signal corresponding to FcRγ in the RBL and mast cells was much stronger (Fig. 4, *lower part*). A faint signal at a m.w. corresponding to FcRγ was also present on the control samples. We then confirmed the association of FcεRIα with FcRγ in these cells by detection of the former after immunoprecipitation with the anti-

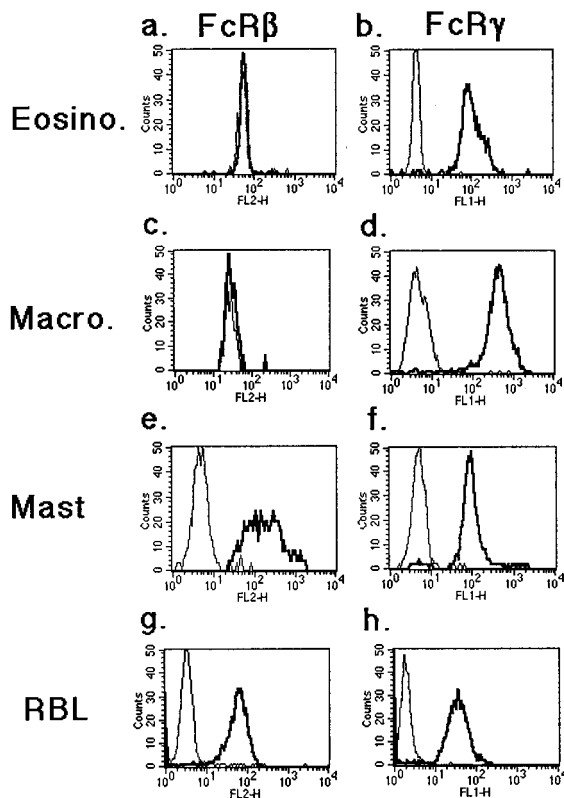


FIGURE 3. Subunit structure of FcεRI on rat eosinophils and macrophages. Flow cytometric analysis of permeabilized cells (a–h). Rat peritoneal cells, purified peritoneal mast cells, and RBL cells were fixed and permeabilized, then incubated with anti-FcεRIα mAb (a, c, e, and g) or anti-Fcγ antiserum (b, d, f, and h) and corresponding fluorochrome-conjugated secondary Abs. Eosinophils (a and b) and macrophages (c and d) were gated. Purified peritoneal mast cells (e and f) and RBL cells (g and h) are shown. Specific staining (thick line) and control isotype or serum (thin line) are indicated.

Fcγ Ab. Indeed, the signal corresponding to FcεRIα was detected as a diffuse band, characteristic form of glycosylated proteins (Fig. 4 upper part) (41). Thus, rat eosinophils and macrophages can express a trimeric αγ₂ FcεRI.

FcεRI expression on eosinophils is up-regulated by IgE in vitro

As in humans, FcεRI expression on eosinophils and macrophages, even after stimulation by thioglycolate injection, appeared relatively low compared with that on mast cells. Since it has been shown that surface expression of the receptor on rat and mouse mast cells was up-regulated by IgE in vitro and in vivo (42–45), we investigated whether IgE was able to increase FcεRI expression on rat eosinophils in vitro. Therefore, peritoneal cells were cultured for 4 days in the presence of IL-5 to prevent eosinophil apoptosis, with or without IgE. Eosinophils were then analyzed by flow cytometry. Cells kept in culture for 4 days without IgE almost completely lost FcεRI expression, as observed for mouse mast cells (45) (Fig. 5a), whereas in the presence of IgE, about 50% of the eosinophil population was expressing FcεRI, albeit at a lower level than freshly isolated cells (Fig. 5b). Thus, ligand up-regulation of FcεRI, or at least prevention of receptor loss, may also occur for rat eosinophils.

FcεRI-mediated ADCC by rat eosinophils and macrophages

We then investigated whether the engagement of the receptor expressed by eosinophils and macrophages could lead to a functional

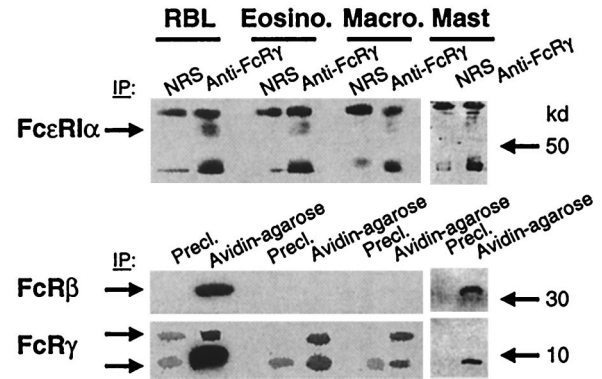


FIGURE 4. Subunit structure of FcεRI on eosinophils and macrophages. Western blot analysis on sorted peritoneal eosinophils and macrophages. *Upper panel*, Sorted cells, purified peritoneal mast cells, and RBL were lysed with digitonin. Sequential immunoprecipitation was performed using normal rabbit serum and anti-Fcγ. Material was run on an 8% nonreducing SDS-PAGE gel, then transferred. Membrane was probed with anti-FcεRIα mAb. *Middle and bottom panels*, Sorted cells, peritoneal mast cells, and RBL were incubated with biotinylated rat IgE. After washing, cells were lysed with digitonin. After preclearing with agarose beads, immunoprecipitation was performed using avidin-agarose beads. Material was run on a 14% reducing gel, then transferred. The upper part of the membrane was probed with anti-FcεRIβ, and the lower one with anti-Fcγ.

response. As previously reported for human eosinophils and macrophages (5, 46), we used ADCC toward *S. mansoni* larvae as the more relevant functional parameter. Peritoneal eosinophils or macrophages, purified from thioglycolate-injected rats, were incubated with schistosomula and serum from *S. mansoni*-infected rats containing anti-schistosoma IgE Abs or with serum from noninfected animals as a negative control. In some samples the role of IgE depletion, anti-FcεRIα mAb, or control mouse IgG was studied. When IgE-containing serum was used, the percentage of cytotoxicity reached 40–75% for eosinophils (Fig. 6a) and 30–92% for macrophages (Fig. 6b). Preincubation of effector cells with anti-FcεRIα Ab led to an inhibition of cytotoxicity ranging, in the different experiments, from 29.5 to 100% (average, 74.1%) for eosinophils (Fig. 6a) and from 42.3 to 57.3% (average, 50.3%) for macrophages (Fig. 6b). Likewise, IgE depletion by incubation of the serum with anti-rat IgE-coated beads led to a 50% inhibition of macrophage cytotoxicity (Fig. 6b). No inhibition was observed

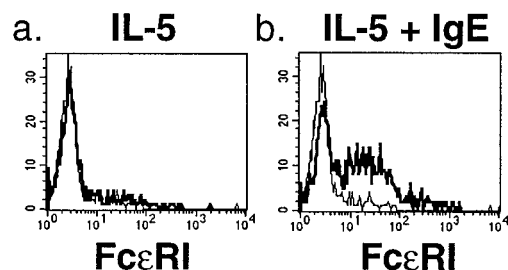


FIGURE 5. IgE-induced up-regulation of FcεRI expression on eosinophils in vitro. Peritoneal cells from thioglycolate-elicited animals were cultured for 4 days with IL-5 in the absence (a) or the presence (b) of rat IgE. Receptor expression on gated eosinophils was detected, after receptor saturation with rat IgE, using biotinylated anti-rat IgE and St-PE (thick line). Biotinylated anti-rat IgE was omitted in the controls (thin line).

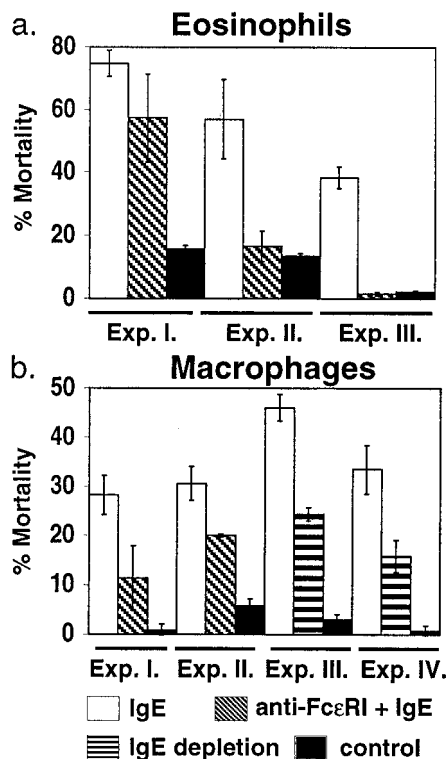


FIGURE 6. Role of FcεRI in IgE-mediated ADCC by eosinophils and macrophages toward *S. mansoni* larvae. Peritoneal eosinophils (a) and macrophages (b) from thioglycolate-injected rats were incubated in duplicate with serum containing 5 μg/ml IgE (□), with normal rat serum (■), with anti-FcεRIα Ab, then with IgE-rich serum (▨) or with IgE-depleted serum (▤). Schistosomula were added 5 h later. Mortality was estimated in three (a) or four (b) independent experiments (I–IV) after 48 h and was expressed as a percentage of dead schistosomula (±SD)

after preincubation with an equivalent amount of normal mouse IgG (not shown). No cytotoxicity was observed when anti-FcεRIα was added to the cells in the absence of IgE-rich serum (not shown). This demonstrates that FcεRI accounts for an important proportion of the IgE-dependent cellular cytotoxicity from eosinophils and macrophages.

Discussion

In this paper, we have shown by flow cytometry, immunofluorescence microscopy, and Western blot analyses that a fraction of thioglycolate-elicited rat macrophages and eosinophils could express FcεRI. This expression could not be mistaken for mast cell expression, since this population accounts for a very low proportion of the peritoneal cells of these treated animals, and since FcεRI expression on mast cells is much higher than that on eosinophils and macrophages. Cell sorting further prevented potential contamination by mast cells. FcεRI expression was detected on neither eosinophils nor macrophages from unstimulated animals, suggesting that receptor expression in cells other than mast cells is very low or absent in resting conditions. This could explain why the receptor was not detected in earlier works, including our studies (31).

As described for mouse mast cells (42, 45) and basophils (43) as well as for rat mast cells (44), receptor expression was up-regulated on eosinophils by incubation with its ligand. A very strong adherence of macrophages to culture wells during the 4-day incubation prevented us from assessing the phenomenon in this cell type. This regulatory mechanism would provide a way for the or-

ganism to optimally respond to IgE-mediated stimulation as it occurs in immediate hypersensitivity reactions or during parasitic infections.

IgE-dependent cytotoxicity toward schistosomula has been reported in previous studies for rat macrophages (29), eosinophils (30), and platelets (19). Experiments using an mAb directed toward human eosinophils and cross-reactive with B cell CD23 suggested that the low affinity IgE receptor, FcεRII/CD23, was mainly involved in this process (31). However, the role of FcεRI was not investigated, since FcεRII/CD23 was for a long time considered to be the only IgE receptor identified on these cell types in both rats and humans. The recent demonstration that FcεRI expressed on human eosinophils or platelets was involved in ADCC (5, 6) led us to re-examine its function in the rat.

In the present study we demonstrate that a subpopulation of rat eosinophils and macrophages expressed a functional FcεRI, involved in IgE-mediated cytotoxicity against *S. mansoni* larvae.

Interestingly, using specific Abs, flow cytometric analyses on permeabilized cells and Western blot experiments failed to reveal the presence of the FcR β-chain in these cell types, while the FcR γ-chain was detected. These results contrast with the previous finding that rat FcR β-chain was required for receptor expression on transfected COS-7 cells (47). Differences between the simian immortalized cell line and freshly isolated rat cells or the presence of a β-like chain in these later might explain this discrepancy. The trimeric α₂ structure of FcεRI expressed by rat eosinophils and macrophages is identical not only with that found on the corresponding human cell types but also with that found on their counterparts in transgenic mice expressing the human FcεRIα under the control of its own promoter region (9, 10). Sequencing and comparison of rat, mouse, and human FcεRIα promoter regions should allow determination of the respective roles played in the three species by FcRβ and by the FcεRIα promoter regions in the determination of FcεRI cellular distribution.

Taken together, these results clearly indicate a different cellular distribution of the high affinity IgE receptor between humans and rats, on one hand, and mice, on the other hand. Further studies are needed to investigate the genomic organization of the promoter regions in each species and their consequences on gene expression. Our findings provide thus a molecular basis to the similarities found between rat and human during *S. mansoni* infection. They also underline that the restricted cellular distribution of FcεRI in mice, in particular its absence on eosinophils and macrophages, hampers the use of such an animal model for an accurate study of IgE- and FcεRI-mediated human pathologies such as allergic reactions. Cellular distribution of FcεRI in animal models commonly used for in vivo studies of allergic reactions, such as guinea pig, rabbit, and dog, would therefore be worth investigating.

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