Identification of the Low Affinity Receptor for Immunoglobulin E on Mouse Mast Cells and Macrophages as FcγRII and FcγRIII

By Fumiyoshi Takizawa, Martin Adamczewski, and Jean-Pierre Kinet

From the Molecular Allergy and Immunology Section, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, Maryland 20852

Summary

In addition to their well characterized high affinity immunoglobulin E (IgE) receptors (FcεRI) mast cells have long been suspected to express undefined Fc receptors capable of binding IgE with low affinity. In this paper, we show that FcγRII and FcγRIII, but not Mac-2, on mouse mast cells and macrophages bind IgE-immune complexes. This binding is efficiently competed by 2.4G2, a monoclonal antibody against the extracellular homologous region of both FcγRII and FcγRIII. Furthermore, IgE-immune complexes bind specifically to FcγRII or FcγRIII transfected into COS-7 cells. The association constants of IgE binding estimated from competition experiments are about $3.1 \times 10^5\ M^{-1}$ for FcγRII, and $4.8 \times 10^5\ M^{-1}$ for FcγRIII. Engagement of FcγRII and FcγRIII with IgE-immune complexes (after blocking access to FcεRI) or with IgG-immune complexes triggers C57.1 mouse mast cells to release serotonin. This release is inhibited by 2.4G2, and at maximum, reaches 30–40% of the intracellular content, about half of the maximal release (60–80%) obtained after FcεRI engagement. These data demonstrate that mouse FcγRII and FcγRIII are not isotype specific, and that the binding of IgE-immune complexes to these receptors induces cell activation.

The molecular cloning of IgE-receptors has helped to classify these receptors into two very different families of proteins. The high affinity IgE receptor (FcεRI)[1] is a noncovalent tetrameric complex of an α chain, a β chain, and two disulfide-linked γ chains (1, 2). The high affinity binding site of FcεRI is on the α chain, a structure homologous to other Fc receptors and a member of the Ig superfamily. By contrast, low affinity IgE-binding structures are part of the lectin-like receptor family. One of these, FcεRII or CD23, belongs to the calcium-dependent class of animal lectins (3, 4) and the other, Mac-2, also called CBP35 and εBP (5, 6), is of the thiol-dependent class of animal lectins.

The tissue distribution and function of these receptors are also very different. The high affinity IgE receptor is found on the surface of mast cells, basophils (1), and Langerhans cells (7). Crosslinking of this receptor via IgE and antigen results in release of the mediators that cause the symptoms of allergic diseases. CD23 in humans is expressed on a wide variety of hemopoietic cells, among them B and T cells, but in mice, the expression of CD23 appears to be restricted to B cells (3). The role of CD23 is not yet completely understood, but it has been proposed to regulate IgE synthesis, to endocytose IgE-immune complexes in B cells, and to serve as an adhesion molecule. Mac-2 was originally described as a macrophage cell surface marker (8), but it is also expressed in mast cells and fibroblasts (5, 6). In spite of the lack of a typical transmembrane domain, a proportion of Mac-2 molecules in these cells are attached to the cell surface. The mechanism of membrane anchoring may involve binding to cell surface glycoconjugates, because incubation of cells with lactose disrupts the attachment (9). In solution, Mac-2 binds IgE, but this binding is carbohydrate dependent and has not been demonstrated for surface-expressed molecules, so its physiological relevance is at best speculative.

More than a decade ago, two molecules with different molecular weight were isolated by affinity chromatography on IgE-Sepharose from extracts of rat basophilic leukemia cells and rat mast cells (10, 11). Then rat basophilic leukemia cells were shown to express IgG receptors also capable of binding IgE (12). In fact, these receptors seemed to have a greater affinity for rat IgE than for rat IgG (12, 13). Other reports

[1] *Abbreviations used in this paper:* $F(ab')_2$, antigen binding fragment of immunoglobulin molecule obtained by pepsin digestion; Fc, constant fragment of immunoglobulin molecule; FcγRII, low affinity receptor for IgG; FcγRIII, low affinity receptor for IgG; FcεRI, high affinity receptor for IgE; and FcεRII, low affinity receptor for IgE.

indicated that rat IgE could inhibit the binding of IgG to rat lymphocytes (14) or to rat macrophages (15). However, this inhibition was characterized as unidirectional and was thought to be unique for rat cells (16).

Our original goal was to characterize the putative low affinity IgE/IgG receptor on rodent mast cells by molecular methods. Despite the restriction of earlier studies to the rat system, we decided to use mouse mast cells because antibodies are available against the various structures known to bind IgE or IgG. We found that the two low affinity receptors for IgG (FcγRII/III) known to be expressed on the surface of mouse mast cells (17, 18) and macrophages (19), are also low affinity receptors for IgE.

Materials and Methods

Immunoglobulins. Igs were purchased from the following sources: rabbit IgG and goat anti-rabbit IgG $F(ab')_2$-FITC from Organon Teknika (West Chester, PA); mouse monoclonal anti-Dansyl IgE from Pharmingen (San Diego, CA); mouse anti-rat IgG (Fc-specific) from Jackson Immunoresearch Laboratories (West Grove, PA); and mouse monoclonal IgG2b from Southern Biotechnology Associates (Birmingham, AL). The FITC-labeled anti-mouse FcεRII antibody B3B4 (20) was a gift of Dr. D. H. Conrad (Medical College of Virginia, Richmond, VA). The anti-rat Mac-2 antiserum, which crossreacts with the mouse protein, was a gift of Dr. F.-T. Liu (Scripps Clinic, La Jolla, CA). Mouse monoclonal anti-DNP IgE (H1 DNP-ε-26.82) (21) was purified from ascites as previously described (22) or it was purified by ion exchange chromatography on DEAE-Trisacryl (IBF Biotechnics, Columbia, MD) from culture supernatant of the hybridoma grown in Protein-Free Hybridoma Medium (Gibco BRL, Grand Island, NY) in a Mini Flo-Path bioreactor (Amicon, Beverly, MA). Mouse anti-DNP IgE was labeled with FITC by standard procedures (23). Rabbit anti-mouse IgE (22) and mouse anti-DNP IgG (24) were prepared as described. The rat mAb 2.4G2 (anti-mouse FcγRII and -III) (25) was purified from tissue culture supernatant by Protein-G affinity chromatography (Pharmacia LKB Biotechnology, Piscataway, NJ). To make oligomers, 100 mg/ml rabbit IgG or mouse monoclonal IgE were chemically crosslinked using dimethyl suberimidate (Pierce Chemical Co., Rockford, IL) (12) at a crosslinker/protein molar ratio of 10:1 at 30°C for 3 h. Rabbit IgG dimers were obtained from this preparation by size separation on Sephacryl S-300 HR (Pharmacia LKB Biotechnology). They were radioiodinated using chloramine T as described (23). To prepare immune complexes, 1 mg/ml anti-DNP IgG or anti-DNP IgE were incubated with DNP-albumin (Calbiochem Corp., La Jolla, CA) at molar ratios of 1:1–20:1 at 4°C for 1 h and used without further washing. Monomeric IgG and IgE were separated from aggregates by centrifuging at 130,000 *g* for 45 min in an Airfuge (Beckman Instruments, Inc., Fullerton, CA). Only the upper ¾ of the samples were used.

Cells. COS-7, J774A.1, $P388D_1$, and P815 (American Type Culture Collection, Rockville, MD) were grown in DMEM with 2 mM glutamine, 10% FCS, 100 U/ml penicillin, and 100 μg/ml streptomycin (Biofluids Inc., Rockville, MD) at 37°C in a humidified atmosphere containing 5% CO_2. The mouse mast cell line C1.MC/C57.1 (C57.1) (26), a gift of S. J. Galli (Beth Israel Hospital, Boston, MA), was maintained in DMEM as were the other cell lines, except for the further addition of 5 μM β-ME.

Expression Vectors. cDNAs encoding mouse FcγRII and the α chain of FcγRIII (19) were gifts of J. V. Ravetch (Memorial Sloan-Kettering Cancer Center, New York). The cDNA for the γ subunit common to mouse FcεRI and FcγRIII was previously described (27). The expression vector pCDL-SRα296 (28) was a gift of N. Arai (DNAX Research Institute of Molecular and Cellular Biology, Palo Alto, CA). The cDNAs encoding mouse FcγRII and -III were ligated into the XbaI and SacI sites of pCDL-SRα296, the cDNA encoding the γ subunit was ligated into the EcoRI and SacI sites.

Transfection. Transfections were carried out by the DEAE-Dextran method (29) using 5×10^6 COS-7 cells per 162 cm^2 flask and 10 μg of each cDNA-containing vector. The amount of DNA was adjusted to a total of 30 μg by adding carrier DNA. After 48 h, the cells were harvested with Enzyme Free Cell Dissociation Solution (Specialty Media, Lavallette, NJ). For negative controls, the γ subunit was transfected alone.

Flow Cytometric Analysis. Flow cytometric analyses were performed on a FACScan® (Becton Dickinson & Co., Palo Alto, CA). For binding of monomeric IgE or IgE-immune complexes, 10^5 cells were incubated with 10 μg/ml of monomeric IgE-FITC or immune complexes of IgE-FITC and DNP-albumin at 4°C for 2 h and then washed twice with PBS containing 0.1% BSA and 0.1% NaN_3. For removal of cell surface Mac-2, the same buffer was used with the further addition of 25 mM lactose (8). For binding of oligomeric IgG, the cells were incubated with 10 μg/ml of rabbit IgG oligomer at 4°C for 2 h and washed. Goat anti-rabbit IgG $F(ab')_2$-FITC was used as the second antibody to detect IgG binding to the cells. Unstained cells or the cells stained with the second antibody alone were used as negative controls. For competitive inhibition, cells were preincubated with 50 μg/ml 2.4G2 and/or 50 μg/ml unlabeled IgE at 4°C for 2 h before addition of oligomeric IgG or labeled IgE.

Competition Binding Experiments. Competitive binding of ^{125}I-labeled rabbit IgG dimers and unlabeled monomeric Igs to transfected cells was studied as previously described (12). Briefly, cells transfected with either FcγRII or -III or the γ subunit alone were incubated with 10 μg/ml radiolabeled rabbit IgG dimer (corresponding to 6.7×10^{-8} M IgG molecules) and varying concentrations of unlabeled monomeric mouse monoclonal IgG2b, rabbit IgG, or mouse monoclonal anti-DNP IgE (molar ratios of monomer/dimer 0.6:1–150:1, based on IgG molecules). The cell density was 2×10^7/ml, the buffer was PBS containing 0.2% BSA, and the incubation was at 4°C for 2 h. Cells were centrifuged through phthalate oil (6 volume parts dibutyl phthalate, 4 volume parts bis(ethylhexyl)phthalate) to separate bound from unbound ligand. Percent cell-bound radioactivity was calculated by dividing cpm in the cell pellet by total cpm. Nonspecific binding was determined with transfectants expressing γ alone and subtracted from the percent binding value to give percent specific binding. The degree of inhibition by various monomeric Igs was calculated by setting the specific binding in the absence of inhibitors equal to 0% inhibition. 100% inhibition was defined as absence of specific binding as a consequence of inhibition. Saturation binding assays with increasing amounts of radiolabeled IgG dimers were performed before the competition binding experiments to verify expression of the receptors and to determine optimal conditions.

Serotonin Release. C57.1 cells were cultured with 1 μCi/ml of 5-[1,2-^{3}H(*N*)]-hydroxytryptamine binoxalate ([^{3}H]-serotonin) (DuPont Co., Wilmington, DE) for 16 h. All procedures after labeling were performed in buffer containing 25 mM Na_2Pipes (pH 7.1), 100 mM NaCl, 5 mM KCl, 0.4 mM $MgCl_2$, 1 mM $CaCl_2$, 5.6 mM D-glucose, and 0.1% BSA (30). Cells were saturated with monomeric IgE, 2.4G2, or both at 37°C for 1 h and washed. The cells were challenged by rabbit anti-mouse IgE or multivalent antigen (DNP-albumin) for triggering through FcεRI.

For triggering through FCγRs, receptor-bound 2.4G2 was cross-linked with anti-rat IgG (Fc-specific). When IgE and/or 2.4G2 were used as inhibitors, these inhibitors were resupplemented after saturation and washing, and the cells were challenged by IgG- or IgE-immune complexes. The reaction was stopped after 30 min by placing the tubes on ice and sedimenting the cells for 3 min at 4,000 rpm in a microfuge. Radioactivity was determined separately in the pellet and the supernatant. Percent release was calculated by dividing counts per minute in the supernatant by total counts/min.

Results and Discussion

Cell Surface Receptors on C57.1. Our first goal was to establish unequivocally whether IgE binds to a second site on mast cells other than FcεRI. This, and the identification of the binding site, requires antibodies against cell surface proteins known to bind IgG or IgE. For murine cells, several useful antibodies are available, such as 2.4G2, which binds to the extracellular domains of the highly homologous FcγRII and -III receptors and blocks the binding of IgG (25), or B3B4, which recognizes mouse FcεRII (20). Comparable anti-rat reagents are not known. Therefore, we analyzed the mouse mast cell line C57.1 rather than the rat cell line RBL, which had been studied previously. We first characterized relevant cell surface proteins on this cell line (Table 1). C57.1 is FcεRI$^+$, FcεRII$^-$, FcγRII/III$^+$, and Mac-2$^+$. Mac-2 can be removed from the cell surface by lactose, as has been described for RBL (9). The cell line responds to engagement of FcεRI in the same way as RBL (30a).

2.4G2-inhibitable Binding of IgE-immune Complexes to Mast Cells. To investigate binding of Igs to their surface, C57.1 cells were incubated with monomeric antibody or with immune complexes and analyzed by flow cytometry. Monomeric IgE strongly binds to C57.1 (Fig. 1 *A*). This binding is completely inhibited by excess unlabeled monomeric IgE (50 μg/ml) (Fig. 1 *B*). There is no further inhibition when both excess IgE and 2.4G2 are employed (Fig. 1 *C*). This is consistent with the presence of a single class of high affinity binding sites for IgE on these cells, i.e., FcεRI receptors. IgE-immune complexes also strongly bind to C57.1 (Fig. 1 *D*). In contrast to the binding of monomeric IgE, the binding of immune complexes is not completely inhibited by excess monomeric IgE (Fig. 1 *E*), indicating that IgE-immune complexes do indeed bind to a second site in addition to FcεRI. However, the binding of immune complexes is completely blocked by the combination of excess monomeric IgE and FcγRII/III-specific 2.4G2 (Fig. 1 *F*). This demonstrates that the staining not inhibited by monomeric IgE is due to a cell surface structure recognized by 2.4G2. Further binding, which could be attributed to other structures such as Mac-2, is not detected. These experiments were performed with monoclonal anti-DNP IgE purified from serum-free bioreactor supernatant to ensure there is no contamination with other Ig classes and repeated with a preparation of the same antibody from ascites. No difference between the two preparations was detected. Oligomerized IgG binds to C57.1 (Fig. 1 *G*). It is not surprising that its binding is inhibited by 2.4G2 (Fig. 1 *H*), and also by IgE-immune complexes (Fig. 1 *I*). Thus, on C57.1, IgE and IgG bind to the same low affinity binding site, which reacts with 2.4G2 and is therefore likely an FcγRII/III receptor. These findings confirm those of Segal et al. (12), who described mutually inhibitable low affinity binding of IgE and IgG to the RBL mast cell line, and extend them by pointing towards FcγRII/III as the responsible entity.

Table 1. *Cell Surface Receptors for IgE and IgG on C57.1 Mast Cells*

Surface antigen	Antibody	Staining
FcεRI	IgE (Monomeric)	+ + +
FcεRII	B3B4	−
FcγRII/III	2.4G2	+ +
Mac-2	Antiserum	+

Cells were stained with the antibodies shown above, and analyzed by flow cytometry. Anti-Mac-2 staining could be abolished by incubation of C57.1 with 25 mM lactose in flow cytometry buffer.

Binding of IgE-immune Complexes to Macrophage Cell Lines and to a FcεRI-deficient Mast Cell Line. We investigated whether this low affinity, 2.4G2-reactive binding site was also present on cell types that express FcγRII/III receptors but not FcεRI. J774, a mouse monocyte-macrophage cell line (31), does not bind monomeric IgE (Fig. 2 *A*), but does bind IgE-immune complexes to a site that is blocked by 2.4G2 (Fig. 2 *B*). In the same way, 2.4G2 blocks binding of oligomeric IgG (Fig. 2 *C*). J774 stains with an anti-rat Mac-2 antiserum, which crossreacts with the mouse protein. The staining is abolished by prior incubation of the cells with 25 mM lactose, indicating that Mac-2 binds to cell surface conjugates on J774. However, the binding of IgE-immune complexes to J774 is not affected by this treatment (data not shown). Together with the lack of a third binding site for IgE on C57.1, this observation excludes Mac-2 from the ranks of functional cell surface IgE receptors. The ability of cell surface Mac-2 to bind IgE has also been questioned on theoretical grounds (9), because the protein, which does not have a transmembrane region, apparently attaches to the cell surface by binding a membrane glycoconjugate and cannot bind IgE simultaneously. Essentially the same results were obtained with P388D$_1$ (macrophage-like) (32), and the mastocytoma-like P815 (33) (data not shown), which express FcγRII and -III but not FcεRI (18, 34). This is further evidence that the low affinity binding site for IgE on mast cells and macrophages is an FcγRII/III receptor.

Binding of IgE-immune Complexes to Transfected Low Affinity Fcγ Receptors. So far, the identification of the low affinity binding site for IgE as FcγRII/III rests entirely on its reactivity with 2.4G2. However, if there exists an IgE/IgG receptor unrelated to FcγRII/III which either crossreacts with 2.4G2 or binds this antibody via its Fc part, it would simulate the results we have observed. The problem of Fc-binding can be

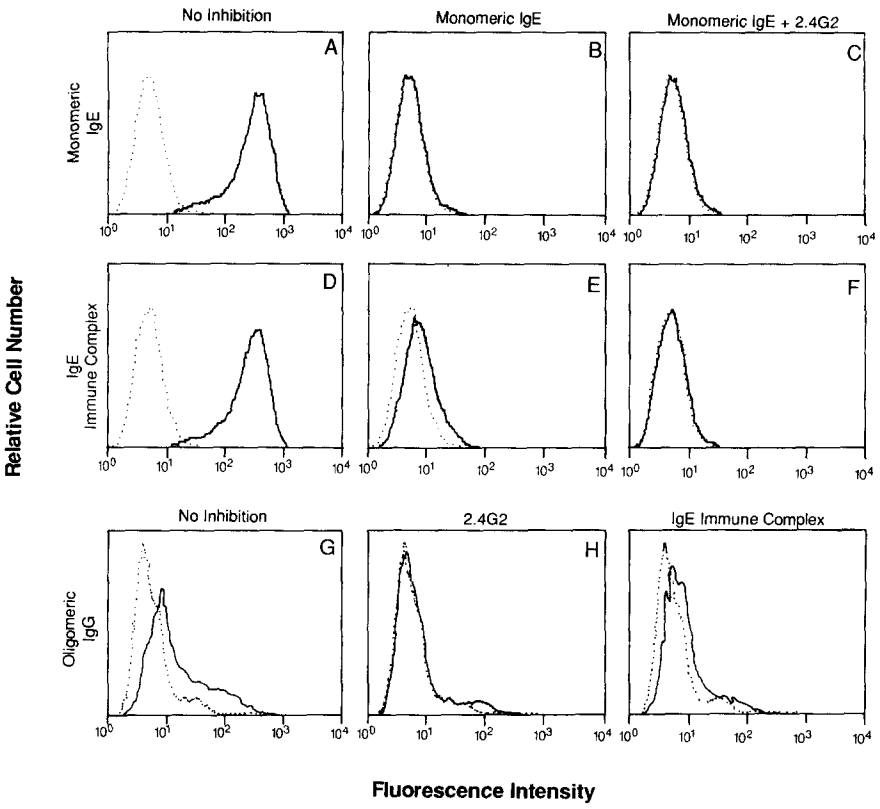

Figure 1. Binding of monomeric IgE, IgE-immune complexes, and oligomeric IgG to C57.1 mast cells. Cells were incubated with FITC-conjugated monomeric IgE (*A–C*), or with FITC-conjugated IgE-immune complexes (*D–F*), or with IgG oligomer followed by FITC-conjugated goat anti-rabbit IgG $F(ab')_2$ (*G–I*). The binding was either not inhibited (buffer control: *A*, *D*, and *G*) or inhibited with unlabeled monomeric IgE (*B* and *E*), or with monomeric IgE and 2.4G2 (*C* and *F*), or with 2.4G2 alone (*H*), or with unlabeled IgE-immune complexes (*I*). Background staining (*dotted lines*) was assessed using no antibody or only the secondary antibody.

circumvented with $F(ab')_2$ fragments of 2.4G2, but even low levels of contaminating whole antibody molecules can interfere (35). Furthermore, the question of crossreactivity is not addressed by this approach. Therefore, we decided to positively identify the receptors by transiently transfecting the genes for the single chain of mouse FcγRII and the two chains (α and γ) required for surface expression of mouse FcγRIII (34) into COS-7 cells. Control cells which were transfected with the γ subunit alone bound neither oligomeric IgG nor IgE-immune complexes (Fig. 3 *E* and *F*). Cells which transiently expressed FcγRII bound IgE-immune complexes (Fig. 3 *B*), as well as oligomeric IgG (Fig. 3 *A*). In both cases the binding was completely inhibited by 2.4G2. A similar staining was observed with cells expressing FcγRIII (Fig. 3, *C* and *D*). This result shows that the so-called IgG receptors FcγRII and -III are not isotype specific; they bind IgE and are thus functional Fcε receptors.

Affinity of FcγRII and FcγRIII for IgE. Because the binding of monomeric antibody to FcγRII/III is too weak to measure, our experiments have relied on the increased avidity of oligomers and complexes of antibodies to visualize binding to the Fcγ receptors. The avidity of such a complex depends on its size, therefore it cannot be used in measurements of affinity constants. In this situation, the relative affinities of different antibodies can be determined by allowing the monomeric antibody to compete with rabbit IgG dimers, whose binding is sufficiently strong to be measurable.

COS-7 cells, which transiently expressed either FcγRII or FcγRIII, were incubated with radiolabeled IgG dimers and either monomeric mouse IgG2b, rabbit IgG, or mouse

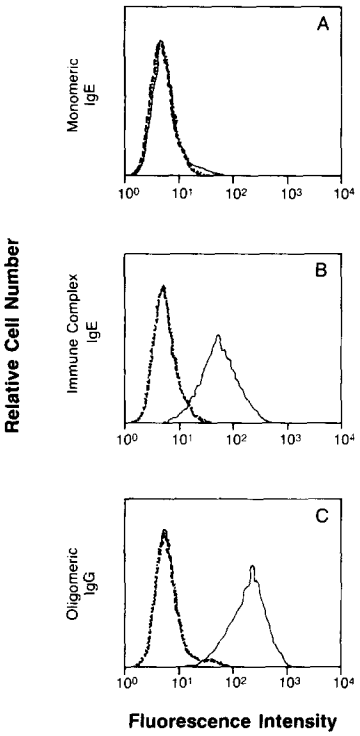

Figure 2. Binding of IgE-immune complexes and oligomeric IgG to J774 macrophages. J774 were incubated with monomeric FITC-labeled IgE (*A*) FITC-labeled IgE-immune complexes (*B*), or rabbit IgG oligomers followed by FITC-conjugated goat anti-rabbit IgG $F(ab')_2$. The binding was not inhibited (buffer control: *solid lines*) or inhibited with 2.4G2 (*dashed lines*). Background staining (*dotted lines*) was assessed using no antibody or only the secondary antibody.

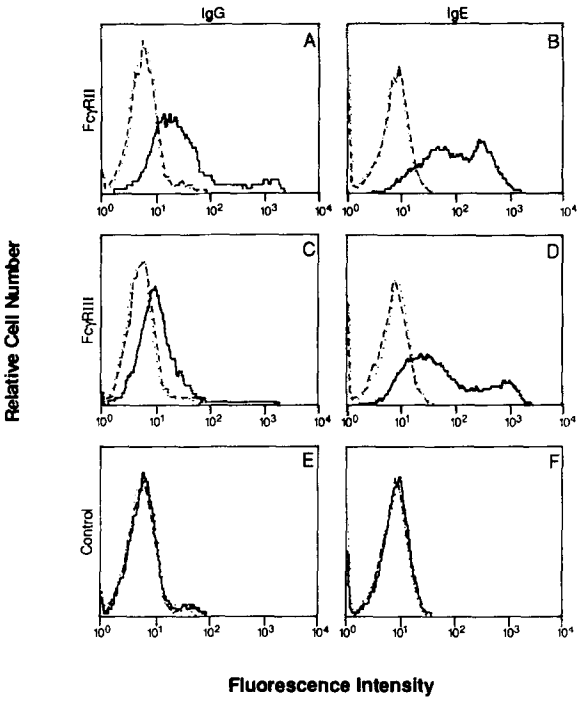

Figure 3. Binding of IgE-immune complexes and IgG oligomers to COS-7 transfectants. COS-7 were transfected with the genes for FcγRII (*A* and *B*), the α and γ subunits of FcγRIII (*C* and *D*), or the γ subunit alone (*E* and *F*). Cells were incubated with rabbit IgG oligomers followed by FITC-conjugated goat anti-rabbit IgG $F(ab')_2$ (*A*, *C*, and *E*) or with FITC-conjugated IgE-immune complexes (*B*, *D*, and *F*). The binding was either not inhibited (buffer control: *solid lines*) or inhibited with 2.4G2 (*dashed lines*). Background staining (*dotted lines*) was assessed using no antibody or only the secondary antibody.

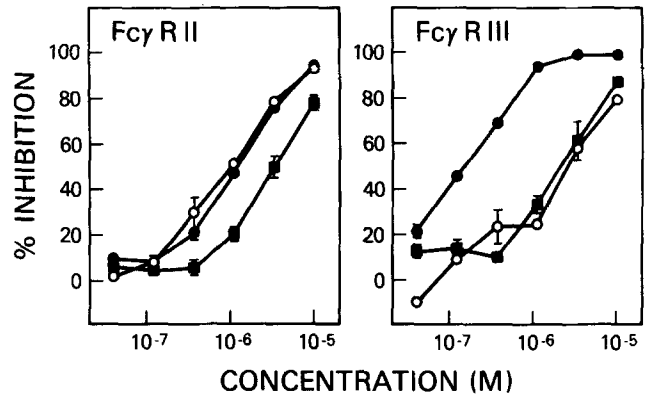

Figure 4. Displacement of IgG dimers from FcγRII and FcγRIII by IgG and IgE. COS-7 were transfected either with FcγRII (*left*) or FcγIII (α and γ subunits, *right*). They were incubated with ^{125}I-labeled rabbit IgG dimers (10 μg/ml, corresponding to 6.7×10^{-8} M IgG molecules) and increasing amounts of monomeric mouse IgG2b (●), monomeric rabbit IgG (○) or monomeric mouse IgE (■). Inhibitors and labeled IgG dimers were premixed before the addition of cells. The degree of inhibition was calculated by setting percent specific binding of IgG dimers without inhibitors equal to 0% inhibition. 100% inhibition was defined as absence of specific binding as a result of competition. Values are means ± SEM. Error bars are omitted where they fall within the size of the data symbol.

Table 2. *Serotonin Release from C57.1 Mast Cells through FcγRII/III or FcεRI*

Preincubation	Challenge	Percent serotonin release
(−)	(−)	0.9 ± 0.2
2.4G2	(−)	1.0 ± 0.1
(−)	Anti-rat IgG	1.2 ± 0.1
2.4G2	Anti-rat IgG	33.2 ± 0.2
(−)	Mouse IgG immune complex	25.2 ± 1.0
2.4G2	Mouse IgG immune complex	1.3 ± 0.4
Anti-Dansyl IgE	(−)	2.9 ± 0.5
(+)	Anti-mouse IgE	1.1 ± 0.2
Anti-Dansyl IgE	Anti-mouse IgE	62.6 ± 1.3

Cells were preincubated with buffer alone, or with 2.4G2 (25 μg/ml), or with monomeric mouse anti-Dansyl IgE (25 μg/ml) and washed, and then challenged by either mouse anti-DNP IgG immune complexes (5 μg/ml IgG), or anti-rat IgG (5 μg/ml), or rabbit anti-mouse IgE (5 μg/ml). Values are means ± SD of triplicate determinations.

IgE. The molar ratio of monomers/dimers (based on IgG molecules) varied between 0.6 and 150. IgE was less efficient than either rabbit IgG or mouse IgG2b at competing with dimers for binding to FcγRII (Fig. 4, *left*), and as efficient as rabbit IgG, but less so than mouse IgG2b, at competing for FcγRIII (Fig. 4, *right*). The approximate association constants for the different monomeric Ig classes are calculated from the reciprocals of the concentration required for half-maximal inhibition of dimer binding. From the inhibition values obtained, we estimate the association constant between FcγRII and mouse IgG2b, rabbit IgG, and mouse IgE as 7.7

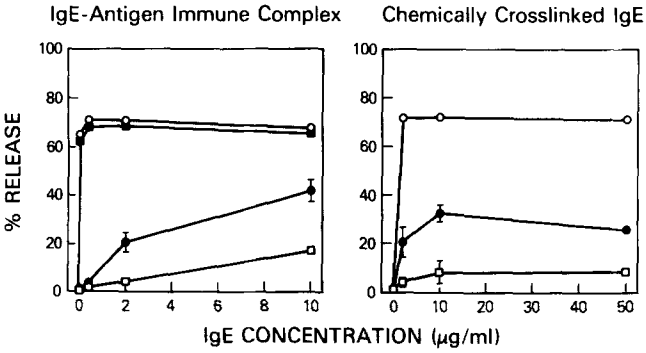

Figure 5. Serotonin release from C57.1 mast cells triggered by IgE-immune complexes or chemically crosslinked IgE. Cells loaded with [^3H]serotonin were triggered by IgE-immune complexes (*left*) or chemically crosslinked IgE (*right*). Triggering was either not inhibited (○), or inhibited by 2.4G2 alone (■), monomeric IgE alone (●), or monomeric IgE and 2.4G2 (□). Values are means ± SD calculated from triplicate samples. Error bars are omitted where they fall within the size of the data symbol.

$\times 10^5\ M^{-1}$, 9.1 $\times 10^5\ M^{-1}$ and 3.1 $\times 10^5\ M^{-1}$, respectively, and for the binding to FcγRIII, 6.7 $\times 10^6\ M^{-1}$, 3.6 $\times 10^5\ M^{-1}$, and 4.8 $\times 10^5\ M^{-1}$. The order of magnitude of these figures agrees well with published values for the interaction between FcγRII/III and IgG (36–38), and with values for the interaction between IgE and the low affinity receptor on mast cells (12), although in these earlier studies differentiation between the subclasses of Fcγ receptors was not possible.

We disagree with Segal et al. (12) on the relative affinities of IgG and IgE for the low affinity receptor. They estimated the affinity of IgE for the low affinity receptor on mast cells to be almost four times as high as that of rabbit IgG, whereas according to our experiments, IgG has the higher affinity. Here it is important to remember that Segal et al. (12) used mast cells for their experiments. Incomplete blocking of FcεRI may account for the difference.

Activation of Mast Cells by IgE through FcγRII/III. To demonstrate that host defences, especially activation of mast cells, can be mediated by IgE through Fcγ receptors, we investigated FcγRII/III-mediated serotonin release from C57.1 cells. First, we established that C57.1 can be activated through FcγRII and -III. This can be shown with IgG-immune complexes (Table 2; 25.2% release, inhibited to 1.3% by prior blocking with 2.4G2) or by crosslinking receptor-bound 2.4G2 with a secondary anti-rat antibody (33.2% release). There is no response to 2.4G2 alone (1.0% release), presumably because it is an insufficient stimulus to crosslink FcγRII/III to mere dimers. Even with optimized crosslinking, the response to FcγR stimulation is much weaker than that to FcεRI stimulation (62.6% release after crosslinking of FcεRI-bound IgE). We do not know whether this reflects merely a difference in receptor number or a difference in the signaling mechanism.

We then investigated whether stimulation through FcγRII/III contributes to activation when C57.1 cells are triggered by immune complexes of monoclonal anti-DNP IgE and DNP-albumin (Fig. 5, *left*) or by chemically crosslinked IgE (Fig. 5, *right*). With both of these triggering agents a comparable plateau of serotonin release (~70%) is reached. 2.4G2 alone does not inhibit release. However, if binding of the IgE-immune complexes to FcεRI is selectively inhibited with monomeric IgE, serotonin release is reduced to about one half of the release observed without inhibition. There is an even greater inhibition when both FcεRI and FcγRII/III are blocked. The magnitude of this further inhibition corresponds well with that of IgG-mediated release through FcγRII/III. IgE can thus trigger mast cells through both FcεRI and FcγRII/III.

To summarize, we have unequivocally shown that: mouse IgE binds with low affinity to a site on mast cells other than FcεRI; that this site corresponds to the mouse IgG receptor FcγRII/III also found on cell types such as macrophages; that the affinity of IgE for the receptors is comparable with that of IgG; and that the binding is functional, because it contributes to mast cell activation. Our goal is now to investigate whether these findings are also relevant to the human system. In that case, our findings would have dramatic implications for explaining the role of IgE in parasitic diseases (39). It might be argued that in view of the serum levels of IgE, binding of IgE to FcγRII or FcγRIII will not occur under physiological conditions, and in any case will be competed by much higher levels of IgG. However, the affinity of these receptors is comparably low for IgG. In fact, normal macrophages are capable of binding IgG-immune complexes because most of the receptors are empty in physiological conditions. It is therefore probable that IgE- as well as IgG-immune complexes will bind to these receptors and activate relevant defence systems, particularly in situ, where the concentration of immune complexes relative to monomeric ligand might be much higher than in the serum.

We are grateful to Dr. S. J. Galli, Beth Israel Hospital, Boston, MA, for the cell line C57.1; to Dr. D. H. Conrad, Medical College of Virginia, Richmond, VA, and Dr. F.-T. Liu, Scripps Clinic, La Jolla, CA, for antibodies; to Dr. J. V. Ravetch, Memorial Sloan-Kettering Cancer Center, New York, for cDNAs for mouse FcγRII and -III; and to Dr. N. Arai, DNAX, Palo Alto, CA, for the vector pCDL-SRα296. We thank Dr. P. Roche for critically reading the manuscript.

M. Adamczewski was supported by the Deutsche Forschungsgemeinschaft, Bonn, Germany.

Address correspondence to Jean-Pierre Kinet, NIH, NIAID, MAIS, Twinbrook II, 12441 Parklawn Drive, Rockville, MD 20852.

Received for publication 15 April 1992.

References

1. Kinet, J.-P. 1990. The high-affinity receptor for IgE. *Current Opin. Immunol.* 2:499.
2. Ravetch, J.V., and J.-P. Kinet. 1991. Fc receptors. *Annu. Rev. Immunol.* 9:457.
3. Conrad, D.H. 1990. FcεRII/CD23: The low affinity receptor for IgE. *Annu. Rev. Immunol.* 8:623.
4. Richards, M.L., and D.H. Katz. 1990. The binding of IgE to murine FcεRII is calcium-dependent but not inhibited by

carbohydrate. *J. Immunol.* 144:2638.
5. Cherayil, B.J., S.J. Weiner, and S. Pillai. 1989. The Mac-2 antigen is a galactose-specific lectin that binds IgE. *J. Exp. Med.* 170:1959.
6. Jia, S., and J.L. Wang. 1988. Carbohydrate binding protein 35. Complementary DNA sequence reveals homology with proteins of the heterogenous nuclear RNP. *J. Biol. Chem.* 263:6009.
7. Wang, B., A. Rieger, O. Kilgus, K. Ochiai, D. Fodinger, J.-P. Kinet, and G. Stingl. 1992. Epidermal Langerhans cells from healthy individuals bind monomeric IgE via FcεRI. *J. Exp. Med.* 175:1353.
8. Ho, M.K., and T.A. Springer. 1982. Mac-2, a novel 32,000 M_r macrophage subpopulation-specific antigen defined by monoclonal antibodies. *J. Immunol.* 128:1221.
9. Frigeri, L.G., and F.-T. Liu. 1992. Surface expression of functional IgE binding protein, an endogenous lectin, on mast cells and macrophages. *J. Immunol.* 148:861.
10. Conrad, D.H., and A. Froese. 1978. Characterization of the target cell receptor for IgE. III. Properties of the receptor isolated from rat basophilic leukemia cells by affinity chromatography. *J. Immunol.* 120:429.
11. Froese, A. 1980. The presence of the two kinds of receptors for IgE on rat mast cells. *J. Immunol.* 125:981.
12. Segal, D.M., S.O. Sharrow, J.F. Jones, and R.P. Siraganian. 1981. Fc(IgG) receptors on rat basophilic leukemia cells. *J. Immunol.* 126:138.
13. Kepron, M.R., H. Bazin, and A. Froese. 1988. The interaction of IgG subclasses with solubilized Fc receptors of rat basophilic leukemia cells. *Mol. Immunol.* 25:599.
14. Yodoi, J., and K. Ishizaka. 1979. Lymphocytes bearing receptors for IgE. III. Transition of FcγR(+) cells to FcεR(+) cells by IgE. *J. Immunol.* 123:2004.
15. Boltz-Nitulescu, G., H. Bazin, and H.L. Spiegelberg. 1981. Specificity of Fc receptors for IgG2a, IgG1/IgG2b, and IgE on rat macrophages. *J. Exp. Med.* 154:374.
16. Spiegelberg, H. 1984. Structure and function of Fc receptors for IgE on lymphocytes, monocytes, and macrophages. *Adv. Immunol.* 35:61.
17. Katz, H.R., J.P. Arm, A.C. Benson, and K.F. Austen. 1990. Maturation-related changes in the expression of FcγRII and FcγRIII on mouse mast cells derived in vitro and in vivo. *J. Immunol.* 145:3412.
18. Benhamou, M., C. Bonnerot, W.H. Fridman, and M. Daëron. 1990. Molecular heterogeneity of murine mast cell FcγR receptors. *J. Immunol.* 144:3071.
19. Ravetch, J.V., A.D. Luster, R. Weinshank, J. Kochan, A. Pavlovec, D.A. Portnoy, J. Hulmes, Y.-C.E. Pan, and J.C. Unkeless. 1986. Structural heterogeneity and functional domains of murine immunoglobulin G Fc receptors. *Science (Wash. DC).* 234:718.
20. Rao, M., W.T. Lee, and D.H. Conrad. 1987. Characterization of a monoclonal antibody directed against the murine B lymphocyte receptor for IgE. *J. Immunol.* 138:1845.
21. Liu F.-T., J.W. Bohn, E.L. Ferry, H. Yamamoto, C.A. Molinaro, L.A. Sherman, N.R. Klinman, and D.H. Katz. 1980. Monoclonal dinitrophenyl-specific murine IgE antibody: preparation, isolation, and characterization. *J. Immunol.* 124:2728.
22. Holowka, D., and H. Metzger. 1982. Further characterization of the β-component of the receptor for immunoglobulin E. *Mol. Immunol.* 19:219.
23. Harlow, E., and D.P. Lane. 1988. Antibodies: A Laboratory Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY. 726 pp.
24. Plotz, P.H., and A. Rifai. 1982. Stable, soluble, model immune complexes made with a versatile multivalent affinity-labeling antigen. *Biochemistry.* 21:301.
25. Unkeless, J.C. 1979. Characterization of a monoclonal antibody directed against mouse macrophage and lymphocyte Fc receptors. *J. Exp. Med.* 150:580.
26. Young, J.D.-E., C.C. Liu, G. Butler, Z.A. Cohn, and S.J. Galli. 1987. Identification, purification and characterization of a mast cell-associated cytolytic factor related to tumor necrosis factor. *Proc. Natl. Acad. Sci. USA.* 84:9175.
27. Ra, C., M.-H.E. Jouvin, and J.-P. Kinet. 1989. Complete structure of the mouse mast cell receptor for IgE (FcεRI) and surface expression of chimeric receptors (rat-mouse-human) on transfected cells. *J. Biol. Chem.* 264:15323.
28. Takebe, Y., M. Seiki, J.-I. Fujisawa, P. Hoy, K. Yokota, K.-I. Arai, M. Yoshida, and N. Arai. 1988. SRα promoter: an efficient and versatile mammalian cDNA expression system composed of the simian virus 40 early promoter and the R-U5 segment of human T-cell leukemia virus type I long terminal repeat. *Mol. Cell. Biol.* 8:466.
29. Sambrook, J., E.F. Fritsch, and T. Maniatis. 1989. Molecular Cloning: A Laboratory Manual. 2nd Edition. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY. 1626 pp.
30. Hultsch, T., J.L. Rodriguez, M.A. Kaliner, and R.J. Hohman. 1990. Cyclosporin A inhibits degranulation of rat basophilic leukemia cells and human basophils. Inhibition of mediator release without affecting PI hydrolysis or Ca^{2+} fluxes. *J. Immunol.* 144:2659.

30a. Adamczewski, M., R. Paolini, and J.-P. Kinet. 1992. Evidence for two distinct phosphorylation pathways activated by high affinity IgE receptors. *J. Biol. Chem.* In press.

31. Ralph, P., and L. Nakoinz. 1975. Phagocytosis and cytolysis by a macrophage tumour and its cloned cell line. *Nature (Lond.).* 257:393.
32. Koren, H.S., B.S. Handwerger, and J.R. Wunderlich. 1975. Identification of macrophage-like characteristics in a cultured murine tumor line. *J. Immunol.* 114:894.
33. Warner, N.L., and Z. Ovary. 1972. Immunoglobulin receptors on mouse mast cells. III. Inhibition of rosette formation of mastocytomas and antibody coated erythrocytes by myeloma and antibody immunoglobulins, free or bound to antigen. *Scand. J. Immunol.* 1:41.
34. Ra, C., M.-H.E. Jouvin, U. Blank, and J.-P. Kinet. 1989. A macrophage Fcγ receptor and the mast cell receptor for IgE share an identical subunit. *Nature (Lond.).* 341:752.
35. Spruyt, L.L., M.J. Glennie, A.D. Beyers, and A.F. Williams. 1991. Signal transduction by the CD2 antigen in T cells and natural killer cells: requirement for expression of a functional T cell receptor or binding of antibody Fc to the Fc receptor, FcγRIIIA (CD16). *J. Exp. Med.* 174:1407.
36. Unkeless, J.C., and H.N. Eisen. 1975. Binding of monomeric immunoglobulins to Fc receptors of mouse macrophages. *J. Exp. Med.* 142:1520.
37. Segal, D.M., and E. Hurwitz. 1977. Binding of affinity cross-linked oligomers of IgG to cells bearing Fc receptors. *J. Immunol.* 118:1338.
38. Segal, D.M., and J.A. Titus. 1978. The subclass specificity for the binding of murine myeloma proteins to macrophage and lymphocyte cell lines and to normal spleen cells. *J. Immunol.* 120:1395.
39. Hagan, P., U.J. Blumenthal, D. Dunn, A.J.G. Simpson, and H.A. Wilkins. 1991. Human IgE, IgG4 and resistance to reinfection with *Schistosoma haematobium*. *Nature (Lond.).* 349: 243.