Mast Cells Lacking the High Affinity Immunoglobulin E Receptor are Deficient in FcεRIγ Messenger RNA

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Summary

A population of cells that express mast cell markers, including the membrane protein p161, but that lack expression of the high affinity IgE receptor, FcεRI, can be routinely grown from bone marrow. Ionomycin, but not IgE immune complexes, causes these cells to release serotonin and to express IL-3 and IL-13 mRNA, consistent with their being FcεRI-deficient mast cells. These p161+/FcεRI− mast cells expressed normal amounts of FcεRI α and β chain mRNA, but extremely low levels of FcεRIγ chain mRNA. In addition, this novel mast cell population expressed CD32 chain mRNA, which p161+/FcεRI+ mast cells did not. CD32γ stable transfectants of Abelson-murine leukemia virus-transformed p161+/FcεRI+ mast cells continued to express FcεRI. This strongly suggests that the failure of p161+/FcεRI− mast cells to express IgE receptors was not caused by the presence of CD32γ chain. Transfection of human FcεRIγ cDNA into p161+/FcεRI− mast cells rescued IgE binding. These stable transfectants released serotonin in response to cross-linkage of FcεRI, demonstrating that the molecular defect of p161+/FcεRI− mast cells is indeed the loss of FcεRIγ expression.
Materials and Methods

Immuno~globulins/Reagents. Purified 2.4G2 and FITC-labeled rat anti-mouse IgE were purchased from Southern Biotechnology Associates, Inc. (Birmingham, AL). IgE was purified as described (12a). 125I-labeled IgE was a kind gift from Dr. Henry Metzger (NIAID, NIH, Bethesda, MD). The mast cell-specific mAb K-1 has been previously described (12a). Rabbit anti-mouse CD3e polyclonal antisera was a generous gift from Dr. John O'Shea (National Cancer Institute, Frederick, MD).

Cells. The derivation of the FceRI+ cell lines used in this work has been described previously (12a). Briefly, BALB/c mouse bone marrow cells were cultured in 10% WEHI-3 supernatant for 7-10 d, and cells were sorted on the basis of IgE binding and p161 expression. Populations were maintained in RPMI 1640 medium supplemented with 15% fetal bovine serum, 2 mM l-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, and 1 mM sodium pyruvate, all from Biofluids, Inc. (Rockville, MD) (cRPMI), supplemented with 10% WEHI-3-conditioned medium (cRPMI/WEHI-3CM) and 20 ng/ml mouse stem cell factor (SCF), generously donated by Dr. Tom Huff (Virginia Commonwealth University, Richmond, VA). The mouse mast cell line CFTL-12 has been described previously (13) and was maintained in cRPMI/WEHI-3CM. The mouse T cell line B413 was kindly donated by Dr. Ronald Germain (NIH). The murine B cell lines A20.1 and pBJNEO were the kind gift from Dr. Jean-Pierre Kinet (NIAID, NIH, Rockville, MD). After 4 d, cells were replated in the same medium with 1.2 mg/ml Geneticin. After 10 d, Geneticin concentration was lowered to 0.8 mg/ml. Cells were maintained in this medium for 2 wk, at which time positive wells were expanded.

Reverse-Transcriptase Polymerase Chain Reaction (RT-PCR). RT-PCR was carried out using 500 ng total RNA, specific primers (see below), 1× PCR buffer (Perkin Elmer Corp., Norwalk, CT), 2 mM MgCl2 during a 50-min RT step at 37°C, and 4 mM MgCl2 during a 35-cycle PCR step with an annealing temperature of 5°C below the melting temperature of PCR primers (Tm), as calculated using the (GC/4°C)+ (A/T × 2°C) formula. Annealing was carried out for 15 s, followed by an extension at 72°C for 30 s in a GeneAmp PCR System thermocycler (Perkin-Elmer Corp., Norwalk, CT).

PCR Primers and Sizes of Expected Products.

Mouse FcεRIα: sense, 5' AGGAGCACAGCACATTTGCT; antisense, 5' AAAGCAGCCGTCGTCTCCGCT (477 bp).

Mouse FcεRIγ: sense, 5' ATGATTCAGCCCTGGATCTTG; antisense, 5' AGTCTCATATGCTTCTGCT (233 bp).

Human FcεRIγ: sense, 5' ATGATTCAGCCCTGGATCTTG; antisense, 5' AGCCAAAGAAGAATAGCG (283 bp).

C3δ: sense, 5' AGCCCTGTACCTGAGAGCAA; antisense, 5' GGAACTGAAGAAGAATAGT (506 bp).

IL-3: sense, 5' ACTCCAAGCTTCAATCAG; antisense, 5' GACGTGAGATCTCCG (337 bp).

IL-4: sense, 5' GAATGTACCAGCAGCCATATC; antisense, 5' CTCATCTACAGGAAATCC (385 bp).

IL-13: sense, 5' ACAGCTCCCTGTTCTCTCCTA; antisense, 5' GCTACTTTGGATTTGGTA (370 bp).

Actin: sense, 5' GATGAGCATGCTGTCGCT; antisense, 5' CCTGTATGCCTCTGGTCGTA (440 bp).

FACS® Analysis. Cells were first stained with 10 μg/ml rat anti-mouse FcεRII/III (2.4G2) for 10 min at 4°C, followed by 10 μg/ml mouse IgE for 1 h at 4°C in staining buffer (PBS, 3% FCS, 0.1% sodium azide). Cells were then washed twice and stained with 10 μg/ml FITC-conjugated rat anti-mouse IgE, then washed twice and analyzed in the presence of propidium iodide with a FACScan® (Becton Dickinson & Co., Mountain View, CA). Control samples were stained with 10 μg/ml 2.4G2 and FITC-conjugated rat anti-mouse IgE.

125I-IgE-binding Assay. Cells were incubated at a concentration of 5 × 104/ml in a volume of 0.5 ml with 200 μg/ml unlabeled IgE or medium for 30 min at 37°C, followed by the addition of 5 μg/ml 125I-labeled IgE for 30 min at 37°C. Cells were then centrifuged through dibutyl phthalate oil in triplicate for 2 min at 15,000 rpm. Cell pellets were frozen on dry ice, and the bottoms of tubes containing pellets were cut off and counted in a Beckman gamma counter. Molecules of IgE bound per cell were determined by standard calculations.

Northern Blotting. Total RNA (20 μg) was electrophoresed on a 1% formaldehyde agarose gel and transferred to supported nitrocellulose (Schleicher & Schuell, Inc., Keene, NH). Northern blots were probed with 32P-labeled cDNAs generated by RT-PCR as described above.

Western Blotting. For detection of CD3δ protein in cell extracts, 1 × 106 cells were washed with PBS/1 mM EDTA and resuspended in Triton X-100 lysis buffer (0.05 M Tris, 0.3 M NaCl, 0.5% Triton X-100, 0.4 mM EDTA, 2.5 mM leupeptin, 2.5 mM aprotinin, and 10 mM PMSF (Sigma Chemical Co., St. Louis, MO) and incubated on ice for 10 min. Samples were centrifuged for 10 min at 15,000 rpm in a microcentrifuge, and supernatants were removed and mixed with 2× sample reducing buffer (0.125 M Tris, 20% glycerol, 10% 2-ME, 4.6% SDS, and 1% bromphenol blue (Sigma Chemical Co.). Samples were boiled 5 min before being subjected to SDS-PAGE on a 14% gel and electroblotted to an Immobilon-P membrane (Millipore Corp., Bedford, MA). Blots were probed with a rabbit polyclonal anti-mouse CD3δ antisera, and developed using the enhanced chemiluminescence (ECL) Western blot detection system according to the manufacturer's specifications (Amersham Corp., Arlington Heights, IL).
For solution-binding studies, cells were resuspended at 5 × 10^6/ml in cRPMI/WEHI-3CM, incubated for 1 h at 4°C with IgE or medium, washed twice, resuspended at 5 × 10^6/ml in cRPMI, and stimulated for 5 h at 37°C with nothing, ionomycin (1 μM), or rat anti-mouse IgE (10 μg/ml). Total RNA was harvested using the RNezon procedure (Tel-Test, Friendswood, TX) according to the manufacturer’s specifications.

Serotonin Release Assay. Cells were resuspended at 10^6/ml in cRPMI/WEHI-3CM containing 2 μCi/ml 5-hydroxy(G-3H)-tryptamine creatinine sulfate (Amersham Corp.) for 1 h at 37°C, washed twice, and incubated 1 h at 37°C in cRPMI/WEHI-3CM with or without IgE (25 μg/ml). Cells were then placed in 96-well flat-bottom plates (Costar Corp., Cambridge, MA) at a concentration of 10^5 per well and incubated for 30 min with 5 μM ionomycin or 30 ng/ml DNP coupled to BSA (DNP-BSA), generously donated by Fred Finkleman (U.S. Health Service, Bethesda, MD). Reactions were stopped by the addition of 100 μl cold RPMI followed by centrifugation. Supernatants were harvested and lysates were made using 100 μl lysis buffer (50 mM Hepes, pH 7.5, 0.5% NP-40, 1% SDS, 5 mM EDTA, 50 mM NaCl, 10 mM sodium pyrophosphate, and 50 mM NaF). Supernatants and lysates were counted, and the percent radioactivity in supernatants was calculated. All samples were done in triplicate.

Results

Existence of FcεR-Deficient Mast Cells. We recently reported an mAb, K-1, which identifies a 161,000-D glycoprotein (p161) expressed on mouse mast cells and on some populations of macrophages (12a). During the course of characterizing the cells that express p161, we observed that in bone marrow cell populations cultured in IL-3 for 7–10 d, ~10% of the p161+ cells failed to bind IgE. The p161+/FceR- cells were purified by cell sorting on day 7 and repurified on day 10 of culture in IL-3. A FACS® analysis of staining of such cells with IgE and FITC-anti-IgE illustrates their failure to bind IgE (Fig. 1). In addition to expression of p161, these cells had other characteristics that are of typical of mast cells. They exhibited mast cell morphology, including large metachromatic granules upon staining with alcian blue or toluidine blue, they contained histamine, and they stained with both the anti-FcγRII/FcγRIII antibody 2.4G2 and with antibodies specific for c-kit (12a). The cells used in this study had been maintained in culture with IL-3 for up to 2 yr, and they resembled phenotypically the cell population characterized in detail by Kinzer, C. A., et al. To confirm the lack of IgE binding found by FACS® analysis, the ability of these cells to bind 125I-labeled IgE was measured. As shown in Table 1, they failed to bind detectable amounts of IgE in this assay, under conditions in which 500 receptors per cell could have been detected. By contrast, FceR+ mast cells, prepared in parallel with the FceR- line, bound 22,800 molecules of IgE/cell, only somewhat less than the IL-3–dependent mast cell line CFTL-12 used here as a positive control.

Activation of FcεR-Deficient Mast Cells. To assess the activation potential of the p161+/FceR- cell line, serotonin release by these cells was compared to release by a line of p161+/FceRI+ cells that had been prepared in parallel.

### Table 1. IgE Binding by p161-positive Cells

| Cell Type      | Medium (cpm) | IgE (cpm) | 125I-IgE plus | IgE Molecules bound per cell |
|----------------|--------------|-----------|---------------|------------------------------|
| CFTL-12        |              |           | 2108 ± 63     | 291 ± 23                     | 3.49 × 10^4 |
| p161+/FceR-    |              |           | 1318 ± 24     | 197 ± 14                     | 2.28 × 10^4 |
| p161+/FceP-    |              |           | 282 ± 4       | 276 ± 11                     | <500         |

Cells (2.5 × 10^6) were incubated with 200 μg/ml IgE or medium alone, followed by 5 μg/ml 125I-IgE. Cell pellet was collected after centrifugation through dibutyl phthalate oil, and cpm were determined and used to calculate the number of IgE molecules bound per cell.

Cross-linkage of IgE resulted in activation, in the form of serotonin release only from p161+/FceR+ cells (Fig. 2 A). However, the calcium ionophore ionomycin induced substantial serotonin release from both FceR+ and FceR- mast cells.

Mast cells are known to express and secrete multiple cytokines after FceRI cross-linkage that is increased by the addition of IL-3 (16). We assessed the relative abilities of FceR-deficient and FceR+ mast cells to express cytokine RNA after stimulation with IgE immune complexes. As shown in Fig. 3, IgE immune complexes induced both IL-3 and IL-13 mRNA by p161+/FceR+ cells. However, no detectable mRNA for either IL-3 or IL-13 was detected in FceR-deficient cells in response to IgE cross-linkage. The calcium ionophore ionomycin stimulated expression of cytokine mRNA in these cells to the same extent as in FceR+ cells.

FceRI-deficient Mast Cells have Diminished Expression of FceRIγ. To assess the expression levels of FceRI α, β, and γ chains, RNA extracted from FceR+ or FceR-deficient cells was used in Northern blotting experiments. Expression of mRNA for FceRI α and β chains in FceR-deficient mast
cells was similar to that of FceR+ cells or of the mast cell line CFTL-12 (Figs. 4, A and B). However, FceR-deficient mast cell expression of FceRIγ was undetectable by Northern analysis in several experiments, although it could be detected by RT-PCR using optimized conditions (Fig. 4 C and data not shown). Similar results were obtained with three separate groups of cells. One of these lines was tested after culture for no more than 2 mo. Furthermore, γ message could not be induced by a variety of stimuli, including PMA, ionomycin, PMA and ionomycin, and incubation with IgE immune complexes (data not shown). Since it is known that all three chains of FceRI are required for proper expression (17), this major decrease in FceRIγ mRNA expression could explain the lack of IgE binding and of IgE-mediated serotonin release, as well as cytokine production by FceRIγ-deficient cells.

p161-positive Macrophages Fail to Express FceRIα. Since the K-1 antibody also recognizes some macrophage populations, we assessed the expression of the FceRIα chain in such p161+ macrophages. Macrophages were derived from cultures of mouse bone marrow cells that had been grown in CSF-1 or GM-CSF, both of which express p161 (12a). These cells failed to express detectable mRNA for FceRI α chain, as detected by RT-PCR (Fig. 5). As expected, mast cell isolates, including the p161+ /FceR-deficient cells, expressed easily detectable amounts of this message. These results, coupled with antibody staining and morphological assessments, strongly support the conclusion that the p161+ /FceR-deficient cells are of mast cell lineage, not macrophage lineage. In view of this and of the finding that they are deficient in a chain involved in the expression of FceRI, we will denote this population as "FceRI-deficient mast cells."

Expression of CD3γ in FceRI-deficient Mast Cells. It is known that the γ chain of CD3 is homologous to FceRIγ (18), and that the γ chain can appear in CD3 in place of or in combination with 3 chain (19-21). Although human CD3γ can substitute for FceRIγ in assembly of FceRI (22) or FcγRIII (10), mouse CD3γ is unable to yield functional expression of FcγRIII in COS-7 cells (10). In an effort to assess expression levels of FceRIγ-related proteins in the p161+ /FceRI-deficient mast cells, we carried out RT-PCR for CD3γ on total RNA derived from these cells and from p161+/FceRI+ cells. As anticipated from previous reports that mouse mast cells do not express CD3γ chain (20), p161+/FceRI+ mast cells were negative by RT-PCR for γ. Surprisingly, CD3γ mRNA was clearly detectable in the FceRI-deficient mast cells by RT-PCR (Fig. 6). Similar results were obtained in multiple experiments with separate cell isolates. Despite the fact that CD3γ mRNA was detectable by RT-PCR, we were unable to demonstrate it by Northern analysis, nor could we detect CD3γ protein by Western blotting techniques. This suggests that the level of expression of CD3γ in p161+/FceRI-deficient cells is quite low.

CD3γ Transfection Does Not Affect FceRI Expression by FceRI+ Mast Cells. Although it seemed more likely that a decrease in FceRIγ expression was responsible for the lack of FceRI expression, it also remained possible that an abnormal expression of CD3γ in p161+/FceRI-deficient cells might be responsible for this phenotype. To test this possibility, we created stable transfectants of FceRI+ mouse mast...
cells with mouse CD3γ. These cells had previously been transfected with the Abelson murine leukemia virus DNA, resulting in the creation of an IL-3-independent mouse mast cell line. The high proliferative rate of this cell line made it an ideal choice for stable transfection. We obtained several clones that expressed CD3γ, as determined by RT-PCR and Western blotting (Fig. 7 A, and data not shown). FACSγ analysis of IgE binding by these transfectants showed no notable decrease in IgE binding from untransfected cells; in fact, several transfectants showed a slight increase in IgE staining (Fig. 7 B). Thus, it appears that aberrant expression of CD3γ is not responsible for the FceRI-deficient mast cell phenotype.

Reconstitution of FceRIγ Restores IgE Binding and Responsiveness in FceRI- Mast Cells. To prove that the FceRI- phenotype of the p161-positive/FceRI-deficient cells was caused by the observed lack of FceRIγ, we stably transfected the human FceRIγ gene coupled to a neomycin-resistance marker gene into an IL-3-dependent, FceRI-deficient mast cell isolate. A series of neomycin-resistant isolates derived from this transfection were screened for the capacity to bind IgE (Fig. 8). These lines, although clearly capable of binding IgE, appeared to express many fewer receptors than conventional p161+/FceRI+ cells. To demonstrate that these FceRI+ transfectants actually expressed human FceRIγ, we carried out RT-PCR using primers that distinguished between mouse and human γ chain mRNA. Three individual transfectants that were capable of binding IgE were tested for human FceRIγ mRNA by RT-PCR, and all three proved to express this mRNA (Fig. 9 A). RT-PCR for mouse FceRIγ showed little or no mouse γ mRNA expressed by these cells. Indeed, human FceRIγ transfectants and cells transfected with a vector expressing only the neomycin-resistance gene actually appeared to express less mouse γ mRNA than did the parental p161+/FceRIγ-deficient cells (data not shown).

To determine if expression of human FceRIγ also restored responsiveness to FceRI cross-linkage, we tested the human γ transfectants in the serotonin release assay system. Although these transfectants expressed much lower amounts of FceRI than did bone marrow–derived mast cells, they responded quite well to FceRI cross-linkage, showing serotonin release levels that were commensurate with those seen using bone marrow–derived mast cells in previous experiments. Cells that expressed the antibiotic resistance gene alone did not release serotonin (Fig. 10). Similar results were obtained in several experiments using four separate clones expressing human γ chain and seven expressing only the neomycin resistance gene. The complete restoration of this IgE-mediated response indicates that FceRIγ expression alone is able to correct the phenotype of FceRI-deficient mast cells, and that low levels of expression of FceRI are sufficient for substantial release of serotonin.

Figure 4. Expression of FceRI α, β, γ chains in p161+/FceR+ and p161+/FceR- mast cells. Total RNA was harvested from CFTL12, p161+/FceRI+ or p161+/FceR- mast cells or the murine B cell lines A20.1 and O13D5. Northern blots were probed with cDNAs specific for FceRI α (A), β (B), or γ (C). Locations of expected bands are indicated.

Figure 5. p161+ macrophages do not express FceRIγ. Total RNA was extracted from bone marrow–derived macrophages grown in CSF-1 or GM-CSF, from A20.1 B cells, or from p161+/FceR+-, p161+/FceR-, or CFTL12 mast cells. RNA was subjected to RT-PCR using primers specific for FceRI (A), or actin (B). Sizes of expected RT-PCR products are indicated.
Figure 6. FceRI-deficient mast cells express CD3γ. Total RNA was extracted from CFTL-12 mast cells, A20.3 B cells, p161+/FceR− or p161+/FceRI− mast cells, and subjected to RT-PCR using primers specific for CD3γ. Blank sample contained all reactants except RNA. Sizes of expected RT-PCR product is indicated.

Figure 8. Transfection of FceRIγ into FceRI-deficient mast cells restores IgE binding. FACS® analysis of p161+/FceRI− mast cells (A), p161+/FceRI− mast cells (B), or p161+/FceRI− mast cells transfected with human FceRIγ cDNA (C). Cells were stained with mouse IgE and FITC-rat anti-mouse IgE (solid lines) or FITC-rat anti-mouse IgE alone (dotted lines). Data is representative of eight individual FceRIγ transfectants.

Figure 7. Transfection of CD3γ into p161+/FceRI+ mast cells does not inhibited FceRI expression. IL-3-independent p161+/FceRI+ mast cells were transfected with CD3γ as described. (A) Western blot analysis using polyclonal rabbit anti-mouse CD3γ antibodies was performed with total cell lysates from seven CD3γ transfectants, the parental p161+/FceRI+ mast cell line, or B413 murine T cells. Size of the expected protein is indicated. All clones had previously been shown to express CD3γ by RT-PCR analysis (data not shown). (B) FACS® analysis of CD3γ-expressing p161+/FceRI+ clones. p161+/FceRI+ mast cells, either transfected with CD3γ or untransfected (parental), or p161+/FceRI-deficient mast cells were stained with mouse IgE and FITC-rat anti-mouse IgE (solid lines) or FITC-rat anti-mouse IgE alone (dotted lines). FACS® profile of IgE/FITC-anti-IgE-stained parental cells is shown relative to each clone for reference (dotted lines).

Discussion

Mast cells are responsible for immediate hypersensitivity reactions that can be mediated via cross-linking of IgE bound to surface FceRI by cognate antigen. The resulting degranulation and synthesis of cytokines, arachidonic acid metabolites, and other active substances have been widely studied (for review see reference 2). In this work, we have characterized a novel population of murine mast cells that lack detectable FceRI. These cells routinely appear in short term cultures of bone marrow cells in IL-3. They have been isolated on several occasions by cell sorting based on their expression of p161 and lack of binding of IgE.
Analysis of this population showed it to possess many mast cell characteristics, with the obvious exception of IgE binding. As described in more detail elsewhere (12a), FceRI-deficient mast cells maintain their phenotype in IL-3 culture for at least 2 yr. Although we strongly suspect that these cells occur physiologically, based on their routine appearance in short term culture, they have not yet been identified in vivo or immediately ex vivo. Since p161 is expressed on some macrophage populations, particularly on thioglycolate-induced peritoneal macrophages, its expression on cells in vivo will not unambiguously identify them as mast cells. We have purified mast cells from peritoneal cavities of CBA/JCR mice by density gradient centrifugation; all of the p161+ cells bound IgE, indicating that p161+/FceRI- mast cells were not a detectable component of this mast cell population. We have not yet examined other mast cell populations in the detail required to determine whether p161+/FceRI- cells are a major component.

Long-term lines of FceRI-deficient mast cells failed to respond to IgE cross-linkage in two assay systems. These cells could neither release serotonin nor produce cytokines, demonstrating a severe defect in both short- and long-term activation responses. However, FceRI-deficient mast cells did respond to ionomycin, indicating intact signaling cascades and the capacity to release granule contents and to produce cytokines made by other mast cells.

FceRI-deficient mast cells are severely lacking in FceRIγ expression. mRNA for this chain was undetectable by Northern blot analysis, and it could not be induced by stimulation with immune complexes or phorbol esters, with and without calcium ionophores. Also, cells in long-term culture with IL-3, with or without SCF, did not develop γ expression. Studies involving the selective ablation of either FcεRIα or FcεRIγ (9, 23) have demonstrated normal mast cell development in the absence of FceRI expression.

CD3ζ and FceRIγ are known to have been created via gene duplication; both are located on mouse chromosome 1, have similar intron/exon composition, and share 50% protein homology. Both chains are expressed in some cell populations, including T cells and human NK cells (for review see references 24, 25). The coincidental loss of FcεRIγ in FcεRI-deficient mast cells, together with the unexpected expression of CD3ζ, suggests an altered regulation of these two genes in this population. However, expression of CD3ζ does not appear to be responsible for the loss of FceRI in our cell population since transfection of this chain into mast cells bearing FceRI had no notable effect on IgE binding.

Rather, we show that the FceRI-deficient phenotype is in fact caused by the lack of FcεRIγ. Although mouse FcεRIγ may have better complemented the endogenous α and β chains, we chose human FcεRIγ for our transfection studies so that we could clearly demonstrate expression of transfected cDNA. The extremely low level of mouse FcεRIγ mRNA detected by RT-PCR analysis of transfected cells indicates that we have not selected transfectants with upregulated endogenous mouse FcεRIγ or a minor population of FcεRI+ contaminating cells. Furthermore, only cells transfected with the human FcεRIγ cDNA showed expression of this chain by RT-PCR analysis. The increase in IgE binding by cells transfected with human FcεRIγ was modest but clear. More importantly, this degree of expression of FceRI appeared to convey full signaling potential to these cells, as judged by the serotonin release assay, indicating that FcεRIγ alone is sufficient to return an IgE-responsive phenotype to these cells.

This population of FcεRIγ-deficient cells could be a useful model system for the study of both IgE and IgG receptors and the role they play in mast cell activation and signalling. Studies involving targeted disruptions of the FcεRIα and γ genes have fully demonstrated the essential roles played by FcεRI in anaphylaxis (9, 23). More importantly, the characterization of this novel mast cell population may provide critical insight into the understanding of mast cell ontogeny, which is still enigmatic.
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References

1. Huff, T.F., and L.B. Schwartz. 1993. Biology of mast cells and basophils. In Allergy: Principles and Practice. J.E. Middleton, C.E. Reed, N.F. Adkinson, Jr., and J.E. Yunginger, editors. C.V. Mosby Co., St. Louis, MO. pp. 135-168.

2. Metzger, H. 1991. The high affinity receptor for IgE on mast cells. Clin. Exp. Allergy. 21:269-279.

3. Ravetch, J.V., and J.P. Kinet. 1991. Fc receptors. Annu. Rev. Immunol. 9:457-492.

4. Rieger, A., B. Wang, O. Kilgus, K. Ochiai, D. Mauerer, D. Fodinger, J.P. Kinet, and G. Stingl. 1992. Fc epsilon RI mediates IgE binding to human epidermal Langerhans cells. J. Invest. Dermatol. 99:305-325.

5. Bieber, T., H. de la Salle, A. Wollenberg, J. Hakimi, R. Chizzonite, J. Ring, D. Hanau, and C. de la Salle. 1992. Human epidermal Langerhans cells express the high affinity receptor for immunoglobulin E (Fc epsilon RI). J. Exp. Med. 175:1285-1290.

6. Wang, B., A. Rieger, O. Kilgus, K. Ochiai, D. Maurer, D. Fodinger, J.P. Kinet, and G. Stingl. 1992. Epidermal Langerhans cells from normal human skin bind monomeric IgE via Fc epsilon RI. J. Exp. Med. 175:1353-1365.

7. Gounni, A.S., B. Lamkhioued, K. Ochiai, Y. Tanaka, E. Delaporte, A. Capron, J.P. Kinet, and M. Capron. 1994. High-affinity IgE receptor on eosinophils is involved in defence against parasites. Nature (Lond.). 367:183-186.

8. Maurer, D. E., Fiebigger, B. Reininger, B. Wolf-Winisik, M.-H. Jouvin, O. Kilgus, J.P. Kinet, and G. Stingl. 1994. Expression of functional high affinity (FcεRI) on monocytes from atopic individuals. J. Exp. Med. 179:745-750.

9. Dombrowicz, D., V. Flaman, K.K. Brigman, B.H. Koller, and J.P. Kinet. 1993. Abolition of anaphylaxis by targeted disruption of the high affinity immunoglobulin E receptor alpha chain gene. Cell. 75:969-976.

10. Kurosaki, T., and J.V. Ravetch. 1989. A single amino acid in the glycosylphosphatidylinositol attachment domain determines the membrane topology of Fc εR. Nature (Lond.). 342:805-807.

11. Ra, C., M.-H. Jouvin, U. Blank, and J.P. Kinet. 1989. A macrophage Fcγ receptor and the mast cell receptor for immunoglobulin E share an identical subunit. Nature (Lond.). 341:752-754.

12. Takai, T., M. Li, D. Sylvestre, R. Clynes, and J.V. Ravetch. 1994. FcR gamma chain deletion results in pleiotropic effector cell defects. Cell. 76:519-529.

12a. Kinzer, C.A., A.D. Keegan, and W.E. Paul. 1995. Identification of FcεRIα⁺⁺⁺⁺ mast cells in mouse bone marrow cell cultures. Use of a monoclonal anti-p161 antibody. J. Exp. Med. 182:575-579.

13. Brown, M.A., J.H. Pierce, C.J. Watson, J. Falco, J.N. Ihle, and W.E. Paul. 1987. B cell stimulatory factor-1/interleukin-4 mRNA is expressed by normal and transformed mast cells. Cell. 50:809-818.

14. Lee, W.T., and D.H. Conrad. 1986. Murine B cell hybridomas bearing ligand-inducible Fc receptors for IgE. J. Immunol. 136:4573-4580.

15. Pierce, J.H., P.P. Di Fiore, S.A. Aaronson, M. Potter, J. Pumphrey, A. Scott, and J.N. Ihle. 1985. Neoplastic transformation of mast cells by Abelson-MuLV: abrogation of IL-3 dependence by a non-autocrine mechanism. Cell. 41:685-693.

16. Plaut, M., J.H. Pierce, C.J. Watson, J. Hanley-Hyde, R.P. Nordan, and W.E. Paul. 1989. Mast cell lines produce lymphokines in response to cross-linkage of FceRI or to calcium ionophores. Nature (Lond.). 339:64-67.

17. Blank, U., C. Ra, L. Miller, K. White, H. Metzger, and J.P. Kinet. 1989. Complete structure and expression in transfected cells of the high affinity IgE receptor. Nature (Lond.). 337:187-189.

18. Kuster, H., H. Thompson, and J.P. Kinet. 1990. Characterization and expression of the gene for the human Fc receptor γ subunit. Definition of a new gene family. J. Biol. Chem. 265:6448-6452.

19. Vivier, E., N. Rochet, J.P. Kochan, D.H. Presky, S.F. Schlossman, and P. Anderson. 1991. Structural similarity between Fc receptors and T cell receptors. Expression of the gamma-subunit of Fc epsilon RI in human T cells, natural killer cells and thymocytes. J. Immunol. 147:4263-4270.

20. Orloff, D.G., C. Ra, S.J. Frank, R.D.F. Klaussner, and J.P. Kinet. 1990. Family of disulphide-linked dimers containing γ and η chain of the Tcell receptor and the γ chain of Fc receptors. Nature (Lond.). 347:189-191.

21. Koyasu, S., L. D'Adamo, A. Arulananandam, S. Abraham, L. Clayton, and E. Reinherz. 1992. T cell receptor complexes containing FcεRIγ homodimers in lieu of CD3γ and CD3η components: a novel isoform expressed on large granular lymphocytes. J. Exp. Med. 175:203-209.

22. Howard, F.D., H.-R. Rodewald, J.P. Kinet, and E.L. Reinherz. 1990. CD3γ subunit can substitute for the 3' subunit of the Fε receptor type I in assembly and functional expression of the high affinity IgE receptor: evidence for interreceptor complementation. Proc. Natl. Acad. Sci. USA. 87:7015-7019.

23. Takai, T., M. Li, D. Sylvestre, R. Clynes, and J.V. Ravetch. 1994. FεR gamma chain deletion results in pleiotropic effector cell defects. Cell. 76:519-529.

24. Ravetch, J.V., and J.P. Kinet. 1991. Fc receptors. Annu. Rev. Immunol. 9:457-492.

25. Ravetch, J.V. 1994. Fc receptors: rubor redux. Cell. 78:553-560.