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 CD3ζ chain mRNA, which p161$^+$/FcεRI$^+$ mast cells did not. CD3ζ 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 CD3ζ 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.

Mast cells mediate allergic inflammatory responses through the release of vasoactive mediators, chemotactic factors, arachidonic acid metabolites, and cytokines. Release of contents of exocytic granules is induced by a variety of stimulants including calcium ionophores, the anaphylotoxins C5a and C3a, mellitin, substance P, and formyl-methiolated peptides (1). In particular, antigen-mediated cross-linkage of the high affinity receptor for the Fc portion of IgE (FcεRI) and of a receptor for the Fc portion of IgG (FcγRII/III) are potent physiologic stimuli for the release of the preformed mediators and for the synthesis and secretion of cytokines (for review see reference 2).

FcεRI is expressed on mast cells and basophils (for review see reference 3), Langerhans cells (4–6), eosinophils (7), and some monocytes (8). The receptor is a tetramer consisting of an α chain, a β chain, and a dimer of two γ chains. Disruption of the FcεRIα chain gene results in the failure of knockout mice to express FcεRI and to develop systemic or local anaphylaxis in response to challenge with IgE anti-dinitrophenyl (DNP)[1] and DNP–human serum albumin (9). This result demonstrates the dependence of IgE-mediated anaphylactic responses on FcεRI.

The FcεRIγ chain is also a component of FcγRIII and can function with the T cell antigen receptor as part of the CD3 complex. Importantly, the FcεRIγ chain is required for signal transduction and proper assembly and expression of both FcεRI and FcγRIII (10, 11). Ablation of this gene results in loss of expression of both receptors, with concomitant mast cell and macrophage defects (12).

Mast cell ontogeny has yet to be fully elucidated; a major limitation has been the lack of mast cell–specific antibodies. In the course of characterizing a newly derived mAb, K-1, which recognizes an antigen, p161, expressed on mast cells and on some populations of macrophages, we encountered a population of mast cells in short-term bone marrow cultures that lacked surface FcεRI (12a). These FcεRI-deficient mast cells appear to constitute a physiologic cell population since they routinely occur in short term cultures of bone marrow cells in IL-3. They have been propagated in long-term culture.

In this paper, we define the molecular basis for the failure of these cells to express FcεRI. We show that they express normal levels of mRNA for FcεRIα and FcεRIβ chains and that they release serotonin in response to ionomycin, strongly indicating that they are mast cells. However, they have markedly diminished expression of FcεRIγ mRNA. Reconstituting γ chain through transfection restores both FcεRI expression and the capacity to release serotonin in response to IgE receptor cross-linkage, establishing that the failure of this mast cell population to express FcεRI results from its defect in γ chain expression.

[1] *Abbreviations used in this paper:* cRPMI/WEHI-3CM, complete RPMI supplemented with 10% WEHI-3–conditioned medium; DNP, dinitrophenyl; RT-PCR, reverse transcriptase–PCR; SCF, stem cell factor; T_m50, melting temperature of PCR primers.

Materials and Methods

Immunoglobulins/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). ^{125}I-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 CD3ζ polyclonal antisera was a generous gift from Dr. John O'Shea (National Cancer Institute, Frederick, MD).

Cells. The derivation of the FcεRI$^+$ and FcεRI$^-$ mast 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 013D5 have been described previously (14). Mouse splenocytes were prepared by the removal of BALB/c spleens, followed by manual disruption. Bone marrow cells used as positive controls in FcR cross-linking studies were prepared by removing BALB/c mouse femur bone marrow cells, and growing these cells in cRPMI/WEHI-3CM for 6–8 d. Bone marrow–derived macrophages were prepared by culturing BALB/c bone marrow cells in GM-CSF (40 ng/ml) or monocyte colony-stimulating factor (M-CSF) (40 ng/ml) for 10 d.

Expression Vectors. The mouse full-length CD3ζ cDNA in plasmid pFNEO and the human full-length FcεRIγ cDNA in pBJNEO were the kind gift from Dr. Jean-Pierre Kinet (NIAID, NIH, Rockville, MD).

Cell Transfections/Transformation. Abelson transformation of IL-3–dependent mast cells derived from bone marrow cultures was carried out as described (15). For CD3ζ or human FcεRIγ transfection, 3 × 10^7 FcεRI$^+$ or FcεRI$^-$ mast cells, respectively, were electroporated with 20 μg plasmid DNA at 1,500 V using an electroporator (Isco, Lincoln, NE). Cells were grown for 24 h in cRPMI/WEHI-3 CM and 20 ng/ml SCF, then plated in this medium with 2.5 mg/ml Geneticin (GIBCO BRL, Gaithersburg, 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 $MgCl_2$ during a 50-min RT step at 37°C, and 4 mM $MgCl_2$ during a 35-cycle PCR step with an annealing temperature of 5°C below the melting temperature of PCR primers (T_m50), as calculated using the (G/C × 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′ ATGGTCACTGGAAGGTCTGCC; antisense, 5′ GGTTGAGAGCAATAACCCCGT (675 bp).

Mouse FcεRIβ: sense, 5′ AGGAGCAGAGCAGATCTTGCT; antisense, 5′ AAAGCAGCCGTCGTCTTCGGT (477 bp).

Mouse FcεRIγ: sense,5′ ATGATCTCAGCCGTGATCTTG; antisense, 5′ AGTCTCATATGTCTCCTGGCT (233 bp).

Human FcεRIγ: sense, 5′ ATGATTCCAGCAGTGGTCTTG; antisense, 5′ AGCCAAAGAAGAATATGACCG (283 bp).

CD3 ζ: sense, 5′ AGCCCTGTACCTGAGAGCAA; antisense, 5′ GGAACTGAAGAAGACAAATGT (506 bp).

IL-3: sense, 5′ ACTCCAAGCTTCAATCAG; antisense, 5′ GACCATGTAGAATCTCAG (337 bp).

IL-4: sense, 5′ GAATGTACCAGCAGCCATATC; antisense, 5′ CTCAGTACTACGAGTAATCCA (385 bp).

IL-13: sense, 5′ ACAGCTCCCTGGTTCTCTCA; antisense, 5′ GCTACTTCGATTTTGGTATCGG (370 bp).

Actin: sense, 5′ GATGACGATATCGCTGCGCTG; antisense, 5′ CCTGTATGCCTCTGGTCGTAC (440 pp).

FACS® Analysis. Cells were first stained with 10 μg/ml rat anti-mouse FcγRII/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.

^{125}I-IgE–binding Assay. Cells were incubated at a concentration of 5 × 10^6/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 ^{125}I-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 ^{32}P-labeled cDNAs generated by RT-PCR as described above.

Western Blotting. For detection of CD3ζ protein in cell transfectants, 1 × 10^7 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 CD3ζ antisera, and developed using the enhanced chemiluminescence (ECL) Western blot detection system according to the manufacturer's specifications (Amersham Corp., Arlington Heights, IL).

Fc Receptor Cross-linking. 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 RNAzol 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-^{3}H)-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 FcεRI-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$^+$/FcεR$^-$ 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 ^{125}I-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, FcεR$^+$ mast cells, prepared in parallel with the FcεR$^-$ 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$^+$/FcεR$^-$ cell line, serotonin release by these cells was compared to release by a line of p161$^+$/FcεRI$^+$ cells that had been prepared in parallel.

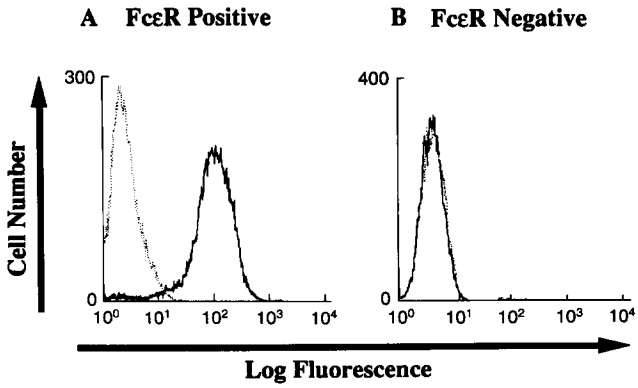

Figure 1. IgE receptor expression by bone marrow–derived mast cells. FACS® purified p161$^+$/FcεR$^+$ (*A*) or p161$^+$/FcεR$^-$ (*B*) mast cells grown in IL-3 for several months were stained with mouse IgE and FITC–rat anti–mouse IgE (*solid lines*) or FITC-rat anti-mouse IgE alone (*dotted lines*).

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

Mast cells are known to express and secrete multiple cytokines after FcεRI cross-linkage that is increased by the addition of IL-3 (16). We assessed the relative abilities of FcεR-deficient and FcεR$^+$ 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$^+$/FcεR$^+$ cells. However, no detectable mRNA for either IL-3 or IL-13 was detected in FcεR-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 FcεR$^+$ cells.

FcεRI-deficient Mast Cells have Diminished Expression of FcεRIγ. To assess the expression levels of FcεRI α, β, and γ chains, RNA extracted from FcεR$^+$ or FcεR-deficient cells was used in Northern blotting experiments. Expression of mRNA for FcεRI α and β chains in FcεR-deficient mast

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

Cell Type	^{125}I-IgE plus Medium (cpm)	^{125}I-IgE plus IgE (cpm)	IgE Molecules bound per cell
CFTL-12	2108 ± 63	291 ± 23	3.49 × 10^4
p161+/FcεR+	1318 ± 24	197 ± 14	2.28 × 10^4
p161+/FcεP-	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 ^{125}I-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.

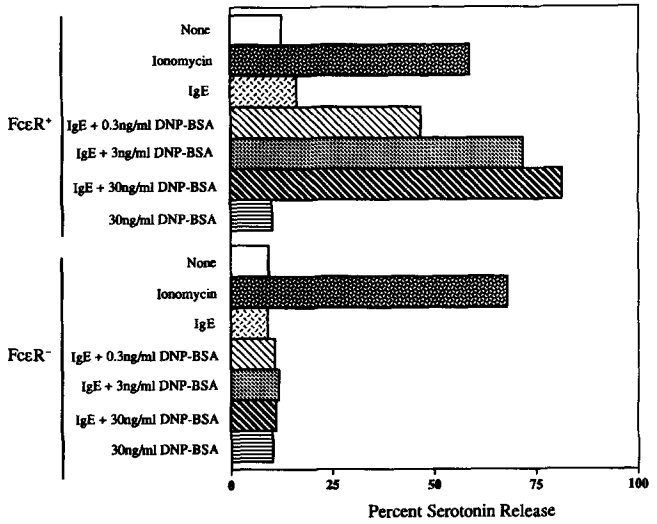

Figure 2. Serotonin release by p161$^+$/FcεR$^+$ or p161$^+$/FcεR$^-$ mast cells. Cells (1×10^6) were pulsed with ^{3}H-5-hydroxytryptamine, incubated with mouse IgE anti-DNP, washed, and stimulated with DNP-BSA. Alternatively, cells were stimulated with 5 μM ionomycin, IgE, or DNP-BSA alone. Data are means of triplicate samples, from one of three representative experiments.

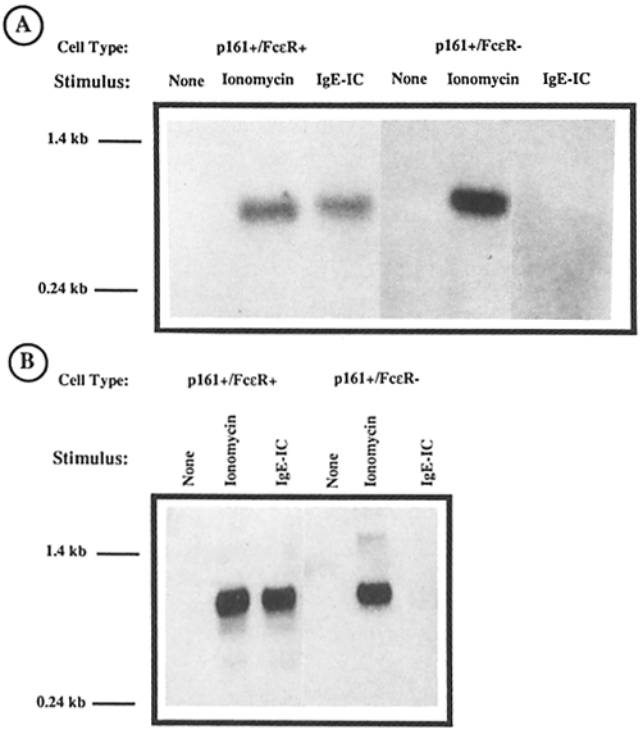

Figure 3. IgE-mediated induction of IL-3 and IL-13 message. p161$^+$/FcεR$^+$ or p161$^+$/FcεR$^-$ mast cells (1×10^7) were incubated with mouse IgE (3 μg/ml), washed and stimulated with 10 μg/ml rat anti-mouse IgE for 5 h. Alternatively, cells were incubated in medium alone and were left unstimulated or stimulated with 1 μM ionomycin. Total RNA was harvested and Northern blots were probed with cDNAs specific for either IL-3 (*A*) or IL-13 (*B*).

cells was similar to that of FcεR$^+$ cells or of the mast cell line CFTL-12 (Figs. 4, *A* and *B*). However, FcεR-deficient mast cell expression of FcεRIγ 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 FcεRI are required for proper expression (17), this major decrease in FcεRIγ mRNA expression could explain the lack of IgE binding and of IgE-mediated serotonin release, as well as cytokine production by FcεRγ-deficient cells.

p161-positive Macrophages Fail to Express FcεRIα. Since the K-1 antibody also recognizes some macrophage populations, we assessed the expression of the FcεRIα 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 FcεRI α chain, as detected by RT-PCR (Fig. 5). As expected, mast cell isolates, including the p161$^+$/FcεR-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$^+$/FcεR-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 FcεRI, we will denote this population as "FcεRI-deficient mast cells."

Expression of CD3ζ in FcεRI-deficient Mast Cells. It is known that the ζ chain of CD3 is homologous to FcεRIγ (18), and that the γ chain can appear in CD3 in place of or in combination with ζ (19–21). Although human CD3ζ can substitute for FcεRIγ in assembly of FcεRI (22) or FcγRIII (10), mouse CD3ζ is unable to yield functional expression of FcγRIII in COS-7 cells (10). In an effort to assess expression levels of FcεRIγ-related proteins in the p161$^+$/FcεRI-deficient mast cells, we carried out RT-PCR for CD3ζ on total RNA derived from these cells and from p161$^+$/FcεRI$^+$ cells. As anticipated from previous reports that mouse mast cells do not express CD3ζ chain (20), p161$^+$/FcεRI$^+$ mast cells were negative by RT-PCR for ζ. Surprisingly, CD3ζ mRNA was clearly detectable in the FcεRI-deficient mast cells by RT-PCR (Fig. 6). Similar results were obtained in multiple experiments with separate cell isolates. Despite the fact that CD3ζ mRNA was detectable by RT-PCR, we were unable to demonstrate it by Northern analysis, nor could we detect CD3ζ protein by Western blotting techniques. This suggests that the level of expression of CD3ζ in p161$^+$/FcεRI-deficient cells is quite low.

CD3ζ Transfection Does Not Affect FcεRI Expression by FcεRI$^+$ Mast Cells. Although it seemed more likely that a decrease in FcεRIγ expression was responsible for the lack of FcεRI expression, it also remained possible that an abnormal expression of CD3ζ in p161$^+$/FcεRI-deficient cells might be responsible for this phenotype. To test this possibility, we created stable transfectants of FcεRI$^+$ mouse mast

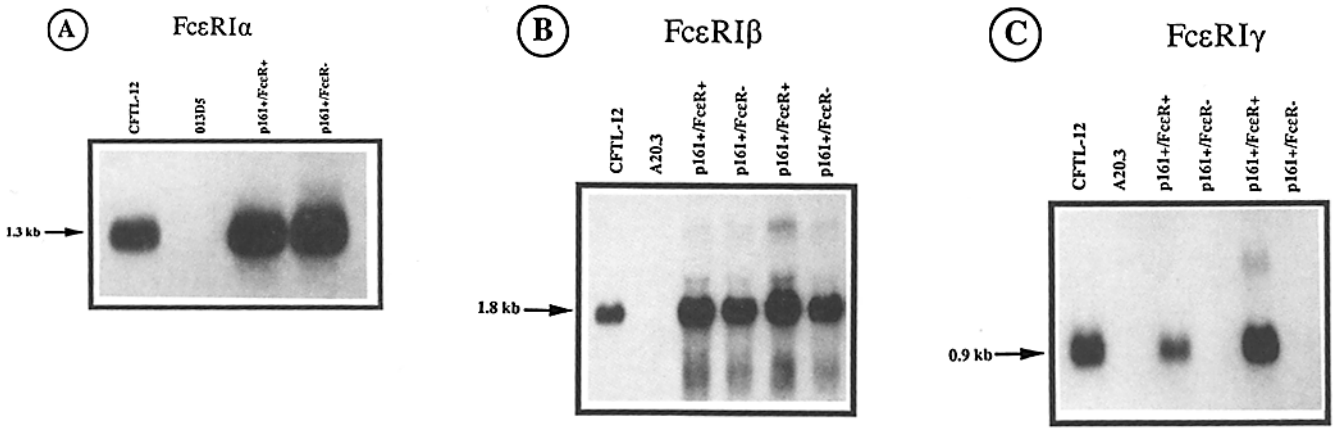

Figure 4. Expression of FcεRI α, β, γ chains in p161$^+$/FcεR$^+$ and p161$^+$/FcεR$^-$ mast cells. Total RNA was harvested from CFTL-12, p161$^+$/FCεRI$^+$ or p161$^+$/FcεR$^-$ mast cells or the murine B cell lines A20.1 and O13D5. Northern blots were probed with cDNAs specific for FcεRI α (*A*), β (*B*), or γ (*C*). Locations of expected bands are indicated.

cells with mouse CD3ζ. 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 CD3ζ, as determined by RT-PCR and Western blotting (Fig. 7 *A*, and data not shown). FACS® 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 CD3ζ is not responsible for the FcεRI-deficient mast cell phenotype.

Reconstitution of FcεRIγ Restores IgE Binding and Responsiveness in FcεRI$^-$ Mast Cells. To prove that the FcεRI$^-$ phenotype of the p161-positive/FcεRI-deficient cells was caused by the observed lack of FcεRIγ, we stably transfected the human FcεRIγ gene coupled to a neomycin-resistance marker gene into an IL-3–dependent, FcεRI-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$^+$/FcεRI$^+$ cells. To demonstrate that these FcεRI$^+$ transfectants actually expressed human FcεRIγ, 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 FcεRIγ mRNA by RT-PCR, and all three proved to express this mRNA (Fig. 9 *A*). RT-PCR for mouse FcεRIγ showed little or no mouse γ mRNA expressed by these cells. Indeed, human FcεRIγ 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$^+$/FcεRIγ-deficient cells (data not shown).

To determine if expression of human FcεRIγ also restored responsiveness to FcεRI cross-linkage, we tested the human γ transfectants in the serotonin release assay system. Although these transfectants expressed much lower amounts of FcεRI than did bone marrow–derived mast cells, they responded quite well to FcεRI 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 FcεRIγ expression alone is able to correct the phenotype of FcεRI-deficient mast cells, and that low levels of expression of FcεRI are sufficient for substantial release of serotonin.

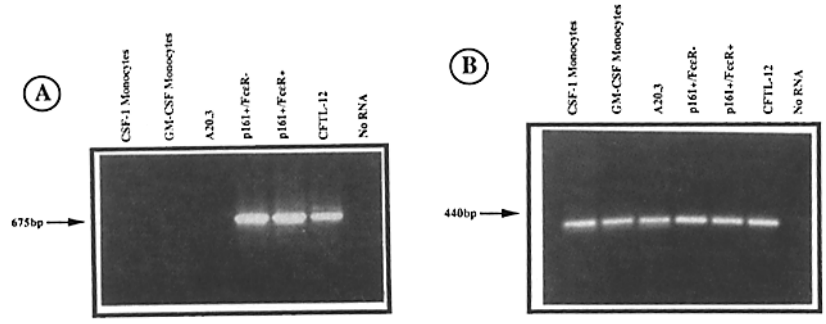

Figure 5. p161$^+$ macrophages do not express FcεRIα. Total RNA was extracted from bone marrow–derived macrophages grown in CSF-1 or GM-CSF, from A20.1 B cells, or from p161$^+$/FcεR$^+$, p161$^+$/FcεR$^-$, or CFTL-12 mast cells. RNA was subjected to RT-PCR using primers specific for FcεRI (*A*), or actin (*B*). Sizes of expected RT-PCR products are indicated.

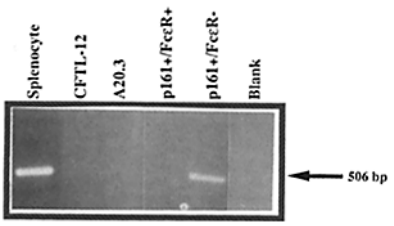

Figure 6. FcεRI-deficient mast cells express CD3ζ. Total RNA was extracted from CFTL-12 mast cells, A20.3 B cells, p161$^+$/FcεR$^+$ or p161$^+$/FcεRI$^-$ mast cells, and subjected to RT-PCR using primers specific for CD3ζ. Blank sample contained all reactants except RNA. Sizes of expected RT-PCR product is indicated.

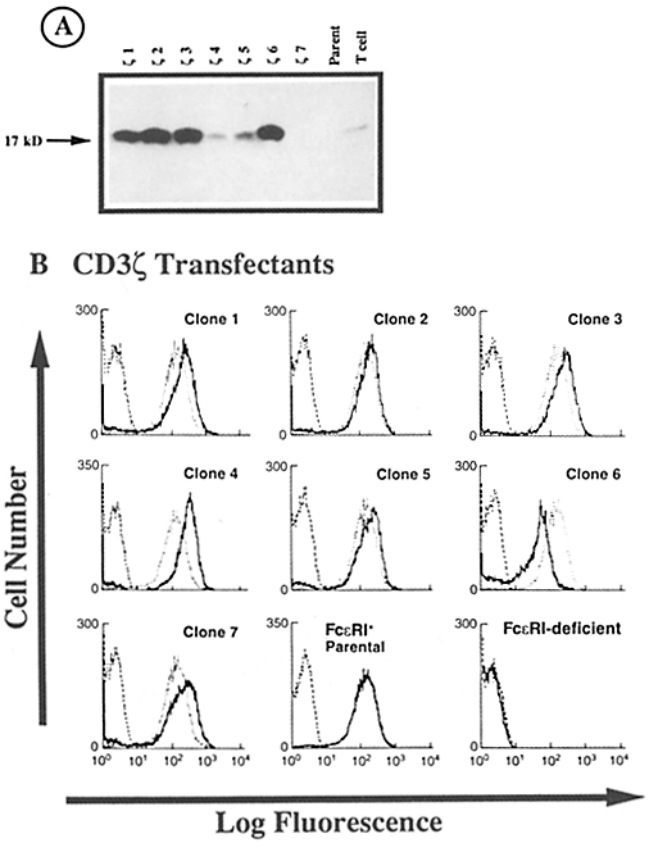

Figure 7. Transfection of CD3ζ into p161$^+$/FcεRI$^+$ mast cells does not inhibited FcεRI expression. IL-3–independent p161$^+$/FcεRI$^+$ mast cells were transfected with CD3ζ as described. (*A*) Western blot analysis using polyclonal rabbit anti-mouse CD3ζ antisera was performed with total cell lysates from seven CD3ζ transfectants, the parental p161$^+$/FcεRI$^+$ mast cell line, or B413 murine T cells. Size of the expected protein is indicated. All clones had previously been shown to express CD3ζ by RT-PCR analysis (data not shown). (*B*) FACS® analysis of CD3ζ-expressing p161$^+$/FcεRI$^+$ clones. p161$^+$/FcεRI$^+$ mast cells, either transfected with CD3ζ or untransfected (parental), or p161$^+$/FcεRI-deficient mast cells were stained with mouse IgE and FITC–rat anti–mouse IgE (*solid lines*) or FITC-rat anti-mouse IgE alone (*dashed lines*). FACS® profile of IgE/FITC–anti-IgE–stained parental cells is shown relative to each clone for reference (*dotted lines*).

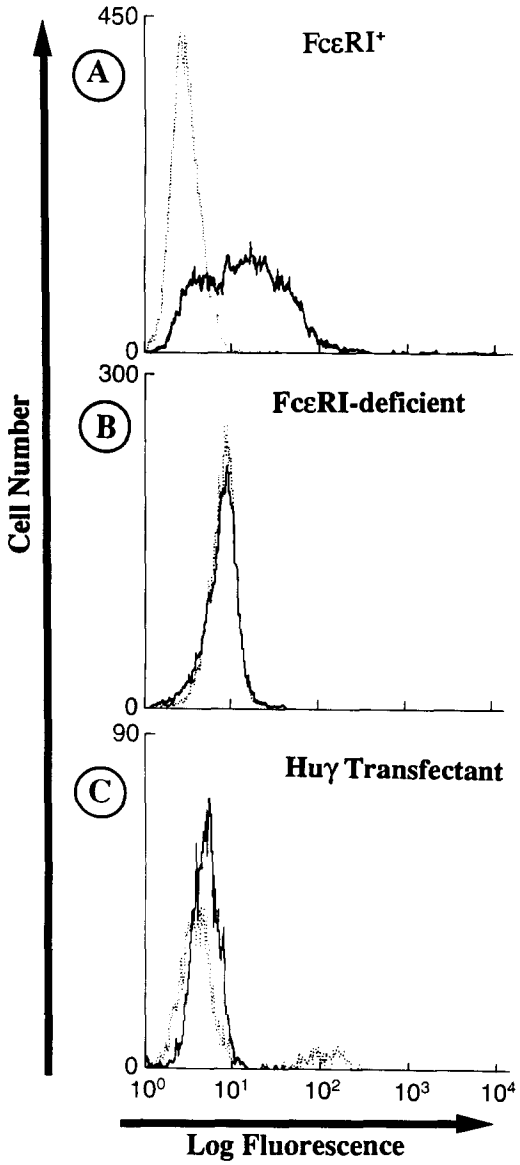

Figure 8. Transfection of FcεRIγ into FcεRI-deficient mast cells restores IgE binding. FACS® analysis of p161$^+$/FcεRI$^+$ mast cells (*A*), p161$^+$/FcεRI$^-$ mast cells (*B*), or p161$^+$/FcεRI$^-$ mast cells transfected with human FcεRIγ cDNA (*C*). Cells were stained with mouse IgE and FITC-rat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

Discussion

Mast cells are responsible for immediate hypersensitivity reactions that can be mediated via cross-linking of IgE bound to surface FcεRI 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 FcεRI. 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.

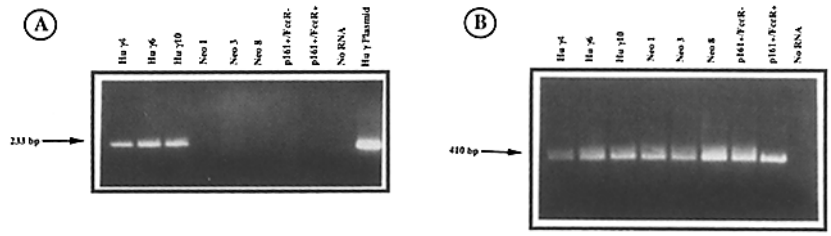

Figure 9. RT-PCR analysis for expression of human FcεRIγ. Total RNA extracted from p161$^+$/FcεRI$^-$ mast cells transfected with human FcεRIγ, neomycin resistance marker, or untransfected cells was subjected to RT-PCR analysis for human FcεRIγ (*A*), or mouse actin (*B*). Plasmid DNA encoding human FcεRIγ was used as a positive control in (*A*). Sizes of expected RT-PCR products are indicated.

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), FcεRI-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$^+$/FcεRI$^-$ 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$^+$/FcεRI$^-$ cells are a major component.

Long-term lines of FcεRI-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, FcεRI-deficient mast cells did respond to ionomycin, indicating intact signaling cascades and

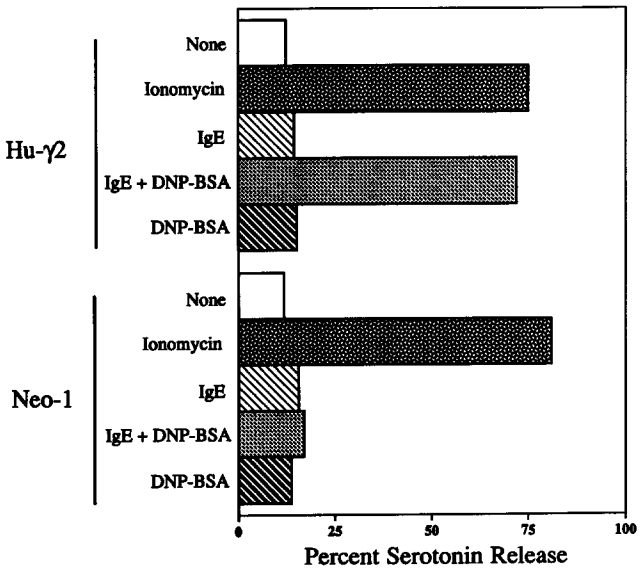

Figure 10. Transfection of FcεRIγ into FcεRI-deficient mast cells restores IgE responsiveness. p161$^+$/FcεRI$^-$ mast cells transfected with human FcεRIγ or neomycin resistance marker were assayed for serotonin release as described in Fig. 2.

the capacity to release granule contents and to produce cytokines made by other mast cells.

FcεRI-deficient mast cells are severely lacking in FcεRIγ 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 FcεRI expression.

CD3ζ and FcεRIγ 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 FcεRI in our cell population since transfection of this chain into mast cells bearing FcεRI had no notable effect on IgE binding.

Rather, we show that the FcεRI-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 FcεRI 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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