

Abolition of Anaphylaxis by Targeted Disruption of the High Affinity Immunoglobulin E Receptor α Chain Gene

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Summary

Mast cells and basophils, which are activated by immunoglobulin E (IgE) and allergen, play a prominent role in anaphylaxis. However, they express at least three types of IgE receptor, including the high affinity IgE receptor (Fc ϵ RI). The relative contribution of these IgE receptors, and possibly other receptors such as Fc ϵ RII/CD23 and Mac-2, to the genesis of *in vivo* anaphylaxis is still unclear. To address this question, we have generated Fc ϵ RI-deficient mice. These mice appear normal and express a normal number of mast cells, but they are resistant to cutaneous and systemic anaphylaxis. These data demonstrate that Fc ϵ RI is necessary for the initiation of IgE-dependent anaphylactic reactions. Therefore, interfering with its function should be an effective means of treating allergy, regardless of the allergen specificity.

Introduction

Many lines of evidence have led to the concept that the interaction between immunoglobulin E (IgE) and mast cells and basophils is the primary effector pathway in allergic responses. Mast cell activation occurs by cross-linking cell surface bound IgE with multivalent allergens, resulting in the release of mediators, such as histamine, which directly produce allergic symptoms (Galli, 1993; Ishizaka, 1989; Serafin and Austen, 1987). Mouse mast cells express at least three types of IgE receptor: the tetrameric high affinity IgE receptor (Fc ϵ RI), which binds monomeric IgE (Kinet, 1992a; Metzger, 1992), and the two low affinity IgG receptors (Fc γ RII and III), which bind both IgG and IgE immune complexes (IgE-ICs and IgG-ICs) (Takizawa et al., 1992). Two other IgE-binding cell surface structures, which are part of the lectin-like receptor family, have been identified: Fc ϵ RII/CD23 (Conrad, 1990) and Mac-2 (CPB35, ϵ BP) (Cherayil et al., 1989; Frigeri and Liu, 1992; Truong et al., 1993). Both are found on a large variety of hematopoietic cells. In addition, Fc ϵ RI is not only expressed on mast cells and basophils, but also on Langerhans cells (Wang et al., 1992; Bieber et al., 1992) and monocytes (Maurer et al., 1993). Thus, there are many hematopoietic cells capable of binding IgE and a variety of IgE-binding structures. The relative role of these IgE

receptors in IgE-mediated allergic reactions is currently unknown.

In addition to mediating degranulation, Fc ϵ RI initiates the release of cytokines, such as interleukin-3, a mast cell growth factor (Burd et al., 1989; Plaut et al., 1989; Wodnar-Filipowicz et al., 1989; Galli, 1993). Fc ϵ RI expression occurs early in ontogeny, at stages in which mast cells are not yet granulated (Thompson et al., 1990). In these precursor cells, Fc ϵ RI cannot mediate degranulation, but it may participate in cytokine secretion and play an as yet undefined role in mast cell development.

To analyze the roles of Fc ϵ RI in developing mast cells and in triggering IgE-dependent allergic reactions, we have generated Fc ϵ RI-deficient mice. Fc ϵ RI is a tetrameric complex of one α , one β , and two disulfide-linked γ chains (Kinet, 1992a; Metzger, 1992). Because only the fully assembled complex is expressed on the cell surface (Blank et al., 1989), targeting one subunit is sufficient to prevent Fc ϵ RI expression. However, γ subunits are part of other Fc receptors and of the T cell antigen receptor (Ravetch and Kinet, 1991) and play a critical role in signal transduction (Kinet, 1992b; Weiss, 1993). Therefore, targeting γ should affect the expression and function of receptors other than Fc ϵ RI. For this reason, and because the receptor binding site is contained entirely in the extracellular portion of α (Hakimi et al., 1990; Blank et al., 1991; Robertson, 1993), we chose to disrupt the α subunit gene. Mice homozygous for the disrupted α chain gene in fact do not express cell surface Fc ϵ RI. Using these mice, we are able to show that Fc ϵ RI does not play a major role in mast cell development, while its presence is necessary to induce local or systemic IgE-mediated anaphylaxis.

Results

Generation of Fc ϵ RI (–/–) Mice

The Fc ϵ RI α subunit is a type I transmembrane molecule with an extracellular segment containing two immunoglobulin-related domains, the second of which is necessary for IgE binding (Robertson, 1993). The Fc ϵ RI α gene is comprised of five exons, with the third and fourth exons encoding the two immunoglobulin domains (Tepler et al., 1989; Ye et al., 1992). A targeting plasmid designed to disrupt the fourth exon by insertion of a neomycin resistance gene was constructed (Figure 1A). Following electroporation with this DNA, D3 and E14TG2a embryonic stem (ES) cells were selected in the presence of G418 and gancyclovir to enrich those that had integrated the vector by homologous recombination. Of the 600 colonies that were isolated and expanded, at least 9 were scored positive for targeted integration of the vector by polymerase chain reaction (PCR) and by Southern blot analysis, demonstrating integration via homologous recombination at the Fc ϵ RI α locus. Hybridization of the same blots with a *neo* probe showed a single copy of the *neo* gene. ES cells from three of these lines were injected into C57BL/6 blastocysts. The chimeras generated from two of these

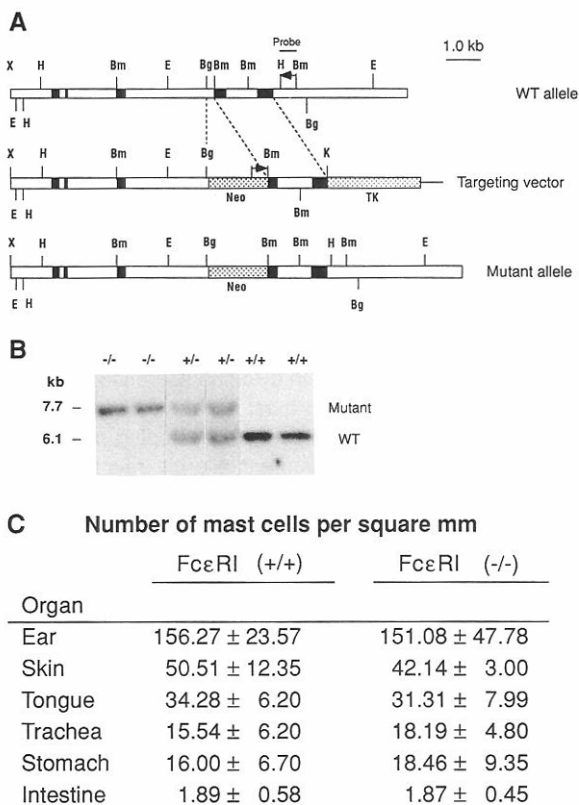


Figure 1. Targeting Vector and Homologous Recombination at the *FcεRIα* Locus

(A) The restriction map of the targeting construct and of the wild-type (WT) and mutated allele is shown. Exons are indicated by closed boxes; *neo* and *tk* cassettes are represented by stippled boxes. Bg, Bm, E, H, K, and X represent restriction sites for BglII, BamHI, EcoRI, HindIII, KpnI, and XhoI, respectively. PCR primers are indicated by arrowheads, and the probe used to detect homologous recombination events is shown.

(B) Southern blot analysis of tail DNA. Hybridizing fragments are 6.1 kb for the wild-type allele (WT) and 7.7 kb for the mutated allele.

(C) Mast cells per square millimeter in ear skin, dermis of back skin, tongue, tracheal lamina propria, submucosa of glandular stomach, and intestinal submucosa. Values are means ± SEM. *FcεRI* (+/+), *n* = 5; *FcεRI* (-/-), *n* = 4.

cell lines transmitted the mutant gene to their offspring (Figure 1B). No overt phenotype was observed in animals that were either heterozygous or homozygous for the mutant gene. Heterozygous and homozygous animals were present in litters at the expected ratios.

Mast Cell Development Appears Normal in *FcεRI* (-/-) Mice

Mast cells from 4- and 8-week-old mice were examined on histological sections of different tissues. No morphological differences between the mast cells of *FcεRI* (-/-) and (+/+) animals of the same litter could be detected. Furthermore, the number of mast cells in six different organs known to be rich in mast cells was equivalent in (-/-) and (+/+) animals (Figure 1C). Thus, the development and differentiation of mast cells does not seem to be affected by the absence of a functional *FcεRIα* gene.

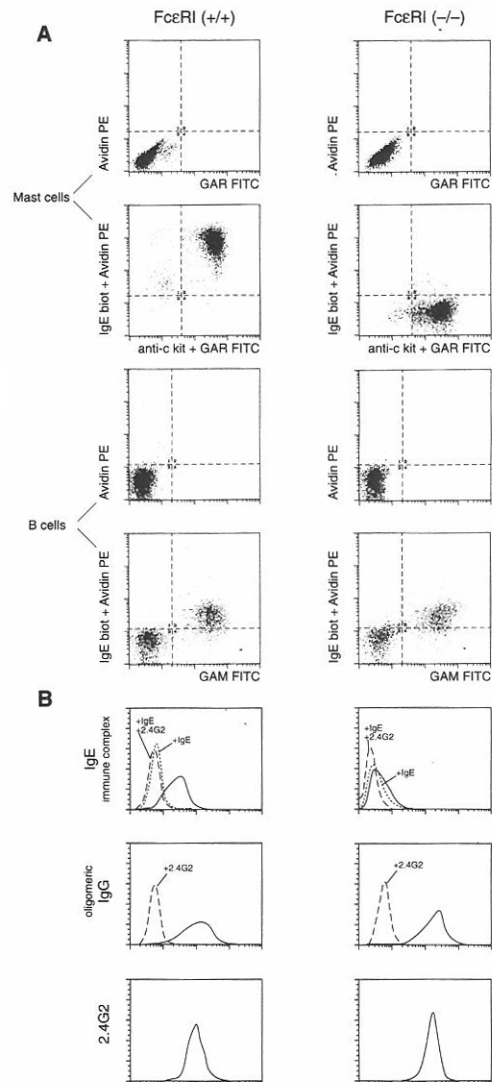


Figure 2. Flow Cytometric Analysis of IgE Binding to BMMCs and B Cells in *FcεRI* (+/+) and *FcεRI* (-/-) Animals

(A) BMMCs were stained with biotinylated monomeric IgE and streptavidin-phycoerythrin and with anti-c-Kit receptor antibody and goat F(ab)₂ anti-rabbit IgG-FITC (top four panels). Splenocytes were stained with biotinylated IgE and streptavidin-phycoerythrin and FITC-labeled goat anti-mouse immunoglobulins (bottom four panels).

(B) Binding of IgE-ICs and oligomeric IgG to FcγRII and FcγRIII on BMMCs of *FcεRI* (+/+) and *FcεRI* (-/-) animals.

Expression of FcγRII/III is shown using 2.4G2-FITC (bottom panels). The binding of oligomeric IgG (center panels) is revealed by using goat F(ab)₂ anti-rabbit IgG-FITC in the absence (solid line) or presence (dashed line) of 2.4G2. Binding of IgE-ICs (top panels) is displayed in the absence (solid line) or presence (dotted line) of monomeric IgE or of both IgE and 2.4G2 (dashed line). The cells analyzed were from a pool of cultured BMMCs obtained from two mice.

Expression of *FcεRI* and *FcεRII/CD23* on Mast Cells and B Cells

To ensure that disruption of the *FcεRIα* gene abolished *FcεRI* expression, we analyzed *FcεRI* expression on bone marrow-derived mast cells (BMMCs). The total number of mast cells from *FcεRI* (-/-) and (+/+) animals after 3 weeks in culture in the presence of interleukin-3 was com-

parable. Those cells were analyzed by fluorescence-activated cell sorting after double staining with anti-c-Kit receptor antibody and monomeric IgE (Figure 2A, top four panels). In both FcεRI (-/-) and (+/+) mice, most of the cells expressed the c-Kit receptor, as expected for mast cells. Binding of monomeric IgE was present in FcεRI (+/+) animals and was absent in (-/-) animals. Therefore, disruption of the FcεRIα gene suppressed FcεRI surface expression and the binding of monomeric IgE.

Spleen B cells constitutively expressing FcγRII/CD23 (Conrad, 1990) also were analyzed by double staining with monomeric IgE and with an antibody against surface immunoglobulins. In both (+/+) and (-/-) mice, the population of B cells appeared normal and virtually all B cells showed IgE binding (data not shown). This binding was due to CD23 because, unlike the binding of monomeric IgE to BMMCs, it was completely inhibited by anti-CD23. Thymocytes were also analyzed for various markers, including TCRαβ, TCRγδ, FcγRII/III, CD5, CD3, CD4, and CD8, and for their capacity to bind monomeric IgE. The thymocytes from both (+/+) and (-/-) mice did not bind IgE, and their various markers of differentiation were qualitatively and quantitatively normal (data not shown).

Increased Expression of FcγRII/III in FcεRI (-/-) Mast Cells

The homologous extracellular domains of FcγRII and FcγRIII react equivalently with the monoclonal antibody 2.4G2 (Ravetch and Kinet, 1991). Therefore, we stained BMMCs with 2.4G2 to detect expression of FcγRII/III (Figure 2B). Unexpectedly, this expression was reproducibly higher in (-/-) mice when compared with the (+/+) littermates. In one representative experiment, the mean and median fluorescence intensity was, respectively, 509 and 469 in (+/+) mice and 561 and 554 in (-/-) mice. This was confirmed by direct binding of oligomeric IgG, which was completely inhibited with 2.4G2.

FcγRII/III on Mast Cells Is an IgE Receptor

We next analyzed the capacity of FcγRII/III to bind IgE-ICs (Figure 2B). In (+/+) mice, IgE-ICs bound principally to FcεRI as shown by inhibition with unlabeled monomeric IgE. However, the addition of 2.4G2 further inhibited the binding of IgE-ICs. This confirms our previous observation that IgE-ICs bind to mouse FcγRII/III (Takizawa et al., 1992). Evidence for this was more striking in (-/-) mice. Monomeric IgE competed poorly with the binding of IgE-ICs. Only 2.4G2 was able to compete efficiently with this binding. Thus, BMMCs of the (-/-) mice bind IgE-ICs via FcγRII/III.

Serotonin Release by Activated BMMCs

BMMCs were loaded with [³H]serotonin, incubated with monomeric anti-dinitrophenyl (DNP) IgE, and then challenged with various amounts of the multivalent antigen DNP[30-40]-human serum albumin (DNP₃₀₋₄₀-HSA). A dose-dependent release of up to 40% of the total cellular serotonin was observed with the BMMCs from (+/+) mice but not from (-/-) mice. However, (-/-) BMMCs were capable of releasing serotonin after stimulation with iono-

mycin and therefore were functionally intact. Engagement of FcγRII/III by cross-linking with 2.4G2 on (+/+) or (-/-) BMMCs did not lead to detectable serotonin release. This result was consistent with previous reports that BMMCs cultured in the presence of interleukin-3 cannot be triggered through FcγRII/III (Katz et al., 1992). However, peri-

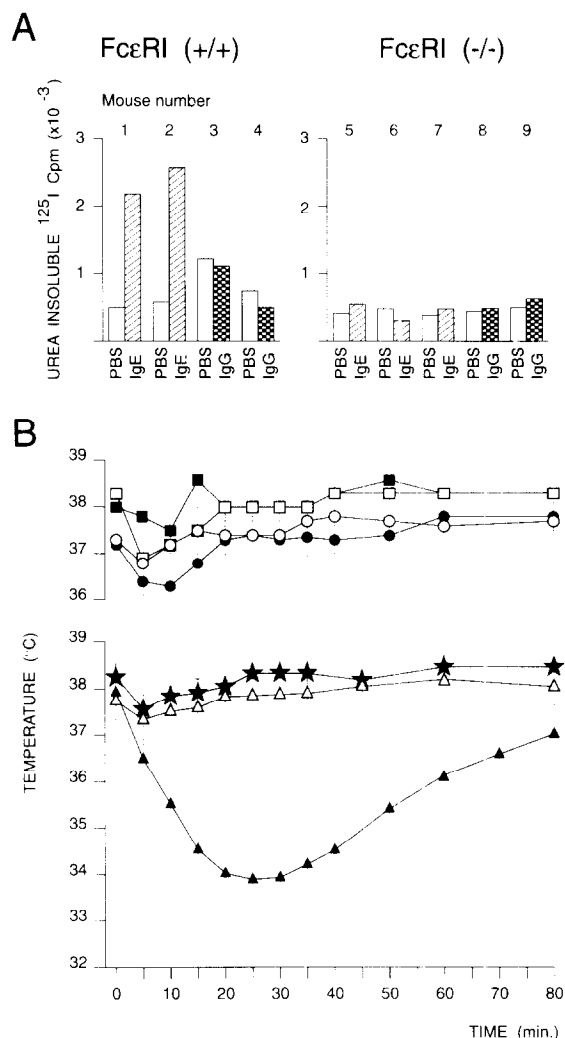


Figure 3. IgE-Mediated Cutaneous and Systemic Anaphylaxis

(A) [¹²⁵I]fibrin deposition in passive cutaneous anaphylaxis in FcεRI (+/+) and FcεRI (-/-) mice. Murine anti-DNP IgE (hatched column) or IgG (checked column) was injected into the right ear and PBS (open column) in the left ear of four FcεRI (+/+) and five FcεRI (-/-) mice, and 20 hr after [¹²⁵I]fibrinogen was injected i.v. After another 30 min, the mice were challenged i.v. with antigen. The urea insoluble fraction was determined 1.5 hr later as explained in Experimental Procedures. (B) Rectal temperature of FcεRI (+/+) and FcεRI (-/-) mice during IgE-induced systemic anaphylaxis. Six FcεRI (+/+) and four FcεRI (-/-) animals (closed and open triangles, respectively) received 20 μg of anti-DNP IgE i.v. One FcεRI (+/+) and two FcεRI (-/-) animals (closed and open squares) received 20 μg of murine anti-DNP IgG. Three FcεRI (+/+) and three FcεRI (-/-) animals (closed and open circles) received 400 μg of rabbit anti-TNP IgG. All the animals received 1 mg of DNP₃₀₋₄₀-HSA in 0.5% Evans blue i.v. 24 hr later. Four additional FcεRI (+/+) mice (closed stars) received antigen only. The monitoring of the rectal temperature was started at the time of antigen injection. Values are means ± SEM.

toneal mast cells from both (+/+) and (-/-) mice could release endogenous histamine after cross-linking of Fc γ RII/III with 2.4G2 (data not shown).

Passive Cutaneous Anaphylaxis Is Prevented in Fc ϵ RI (-/-) Mice

To assess the contribution of Fc ϵ RI in allergic reactions, we first used the *in vivo* model of passive cutaneous anaphylaxis (Wershil et al., 1987), in which local extravasation, fibrin deposition, and tissue swelling are induced by local injection of IgE followed by an intravenous (i.v.) antigenic challenge. Anti-DNP IgE (0.1 μ g) or the same amount of anti-DNP IgG was injected in the right ear of both (+/+) and (-/-) mice. As a control, the left ear of these mice was injected with vehicle alone. After 20 hr, all animals were injected i.v. with [^{125} I]fibrinogen, before being challenged with DNP₃₀₋₄₀-HSA injected with Evans blue dye. The total fibrin deposit and the urea insoluble fraction corresponding to the cross-linked fibrin were quantitated in both ears of each experimental mouse. In (+/+) animals, injection of IgE and antigen led to a fibrinogen/fibrin deposit in the right ear twice as large as the deposit in the left ear. Furthermore, the amount of cross-linked fibrin in the right ear was 4- to 5-fold the amount recovered from the control ear (Figure 3A). In contrast, no differences between the right and left ears were detected in (-/-) mice injected with IgE. No effect was seen in either (+/+) or (-/-) mice injected with IgG. Dye extravasation and swelling (13% weight increase) were detected only in the right ears of (+/+) mice injected with IgE and antigen. Therefore, at the dose of 0.1 μ g, IgE, but not IgG, is capable of inducing passive cutaneous anaphylaxis, and then only when Fc ϵ RI is present.

Fc ϵ RI (-/-) Mice Are Resistant to Systemic Anaphylaxis

Another way to test allergic reactions is to induce systemic anaphylaxis. Here, IgE is injected i.v. 24 hr before i.v. injection of antigen. The antigenic challenge is followed immediately by a dramatic increase in vascular permeability, generalized fluid extravasation, and profound shock exhibited by a drop in blood pressure and body temperature. We first investigated whether the absence of Fc ϵ RI could have an influence on the clearance of IgE from the serum, following an i.v. injection of IgE under conditions similar to those used in the systemic anaphylaxis protocol. With an i.v. injection of 20 μ g of [^{125} I]IgE, the serum clearance over 24 hr appeared similar in both (+/+) and (-/-) mice. The apparent half-life was about 2 hr during the 3 hr following injection and increased to around 8 hr. The data obtained from normal mice were consistent with those from Haba et al. (1985). After 24 hr, there was still a substantial amount (0.7 μ g/ml) of circulating IgE in the serum. Furthermore, comparable levels of radioactivity per milligram of tissue were found in various organs from (+/+) and (-/-) mice. Similar results were obtained when only 1 μ g of [^{125} I]IgE was injected (data not shown). Therefore, at the time of antigenic challenge, the serum level of IgE was similar in both (-/-) and (+/+) mice. This implied that the capacity to form IgE-ICs, which could bind to low

affinity IgE receptors such as Fc γ RII/III, was equivalent in both wild-type and Fc ϵ RI-deficient mice.

To induce systemic anaphylaxis, both (+/+) and (-/-) mice were injected i.v. with 20 μ g of anti-DNP IgE or with vehicle. Another set of mice was injected with 20 μ g of mouse anti-DNP IgG or with 400 μ g of rabbit anti-TNP IgG. All mice were challenged 24 hr later with 1 mg of DNP₃₀₋₄₀-HSA. Evans blue dye was concomitantly injected with antigen to visualize the fluid extravasation. A significant drop in body temperature occurred in the IgE-injected but not in the IgG-injected Fc ϵ RI (+/+) mice (Figure 3B). This drop reached a maximum after about 25 min and was accompanied by obvious tachycardia, piloerection, and prostration. The drop in temperature and other symptoms were completely absent in (-/-) mice. The anaphylactic shock was best visualized by the extravasation of the dye, which was marked in the ears and feet of the Fc ϵ RI (+/+) mice and absent in the Fc ϵ RI-deficient mice (Figure 4).

Discussion

Fc ϵ RI-deficient mice provide a useful tool with which to address questions regarding the physiological role of Fc ϵ RI. The plethora of IgE-binding proteins present *in vivo* and their coexpression on many immune cells previously have made it essentially impossible to determine individual roles for each in IgE-mediated immune responses. Using the Fc ϵ RI-deficient mice we have generated, we characterize the Fc ϵ RI (-/-) phenotype and address questions about the role of Fc ϵ RI in the initiation of anaphylactic responses and in mast cell ontogeny. We also discuss future uses of these mice in defining physiologic protective responses attributable to IgE and Fc ϵ RI.

In mice, the Fc ϵ RI (-/-) phenotype does not appear to include any overt undesirable features. The mice have no obvious propensity for autoimmune diseases or susceptibility to infection, as perhaps would be manifested if their immune systems were altered overtly. The absence of Fc ϵ RI has no apparent effect on B and T cell numbers or on population subsets. The mast cells of these mice express reproducibly higher levels of Fc γ RII/III, but this does not seem to translate into an altered biological response. At present, we have no definite explanation for this increased expression, but it is tempting to speculate that the absence of competition by the Fc ϵ RI α chain is translated into an exclusive availability of γ chains for assembly with Fc γ RIII.

The results of the study on the serum clearance of IgE following i.v. injection were interesting. We had assumed that Fc ϵ RI, as the only high affinity IgE receptor, would act as the principal reservoir for IgE. However, the clearance of IgE in Fc ϵ RI-deficient mice parallels perfectly the pattern observed in normal mice, whether 20 μ g or 1 μ g of IgE is injected. Therefore, Fc ϵ RI would seem to have little or no role in the sequestration of IgE. These data are consistent with the suggestion that Fc ϵ RI/CD23 is the principal binding structure for IgE and affects IgE homeostasis (Chen, 1991).

Some investigators, including us, have speculated

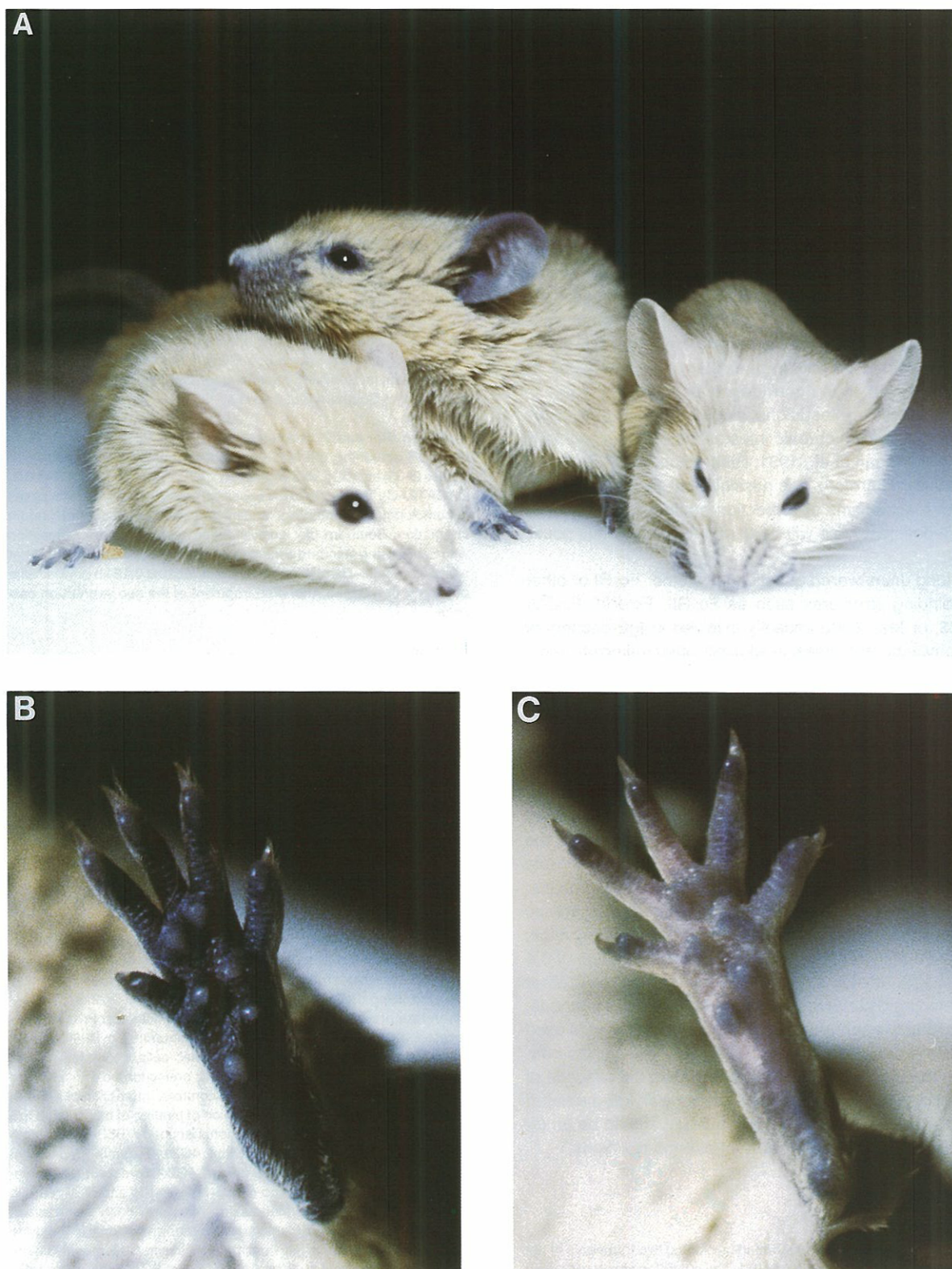


Figure 4. Dye Extravasation in FcεRI (+/+) and FcεRI (-/-) Mice during IgE-Induced Systemic Anaphylaxis

(A) FcεRI (+/+) mouse (middle) and FcεRI (-/-) mouse (left) received 20 μg of anti-DNP IgE i.v. and, 24 hr later, 1 mg of DNP₃₀₋₄₀-HSA in 0.5% Evans blue i.v. (B and C) Feet from FcεRI (+/+) (B) and FcεRI (-/-) (C) mice. As a control, the FcεRI (+/+) mouse (right in [A]) received only 1 mg of DNP₃₀₋₄₀-HSA in 0.5% Evans blue i.v. Photographs of the mice were taken 60 min after the antigen challenge.

about a role for FcεRI in mast cell development. This speculation was based on the early appearance of FcεRI on precursors of mast cells (Thompson et al., 1990; Ashman et al., 1991) and on the capacity of FcεRI to mediate synthesis and release of mast cell growth factors such as interleukin-3. Our data suggest FcεRI does not play an important role in mast cell development. The absence of FcεRI did not affect the number or appearance of mast cells in any of the tissues and organs examined. In addition, mast cells appeared to function normally when stimulated by nonspecific mast cell activators. However, it remains to be seen whether FcεRI will play a role in mast cell proliferation under conditions in which mast cells are chronically stimulated via FcεRI. Similarly, the possible role of FcεRI in immune defense, while not clear at present, may be evidenced only in particular conditions, such as infections. Although the evidence for a protective role of IgE in parasitic infestations is mixed, IgE seems to be protective against chronic infestations of *Schistosoma* in humans (Hagan et al., 1991; Rihet et al., 1991; Dunne et al., 1992). Therefore, it is possible that FcεRI is involved in the mechanisms of protection against parasites. The FcεRI-deficient mice will be useful to analyze this possibility.

A long unanswered question is whether FcεRI or other IgE-binding structures such as FcγRII, FcγRIII, FcεRII/CD23, or Mac-2 are causally involved in IgE-dependent anaphylactic responses. In addition, other unknown mechanisms also could play a critical role. Our results using FcεRI-deficient mice are clear in this regard. The FcεRI-deficient mice are completely protected from IgE-mediated anaphylaxis. This is true despite the fact that these mice express higher levels of FcγRII/III and that experimental conditions were used in which one might expect the formation of IgE-ICs that could activate low affinity IgE-binding proteins. Therefore, FcεRI is necessary for the induction of IgE-mediated anaphylaxis, with a minor role at best for the other lower affinity IgE-binding proteins. The role of IgG-ICs also seems to be minimal, as systemic anaphylactic reactions could not be induced in any mice, either FcεRI (+/+) or (-/-), even after i.v. injection of 0.4 mg of IgG. At this stage we cannot rule out that other IgE-binding proteins may play a role if alternative methods, such as active immunization (Martin et al., 1993), are used to induce antigen sensitivity. However, it is safe to conclude that FcεRI is necessary for initiating the cascade of events leading to the various symptoms of IgE-mediated anaphylaxis. Therefore, targeting FcεRI should be an effective way to prevent or to treat allergic reactions.

Experimental Procedures

Construct

The targeting vector was constructed by cloning two fragments of the mouse FcεRIα into the vector pJNS2. The pJNS2 vector was derived by inserting a 50 bp polylinker between the XbaI and BamHI sites of pNT2 (Tybulewicz et al., 1991). A genomic clone was isolated by screening a 129/Ola genomic λ phage library with mFcεRIα cDNA (Ra et al., 1989). A 1.6 kb BglII-KpnI fragment encompassing exons IV and V was generated by PCR of the genomic clone with primers CAGG-TACCCAGTGTATTGAG and GAAGATCTGGATCCTTGACAT-CAGATGCC and then inserted between the BamHI and KpnI sites of

pJNS2 vector, 3' to the *neo* gene and 5' to the *tk* gene. The second fragment, a 5.5 kb XhoI-BglII fragment including exons I–III was obtained from a BALB/c library genomic clone (Ye et al., 1992), subcloned in pSL301 (In Vitrogen), and then excised by digestion with XhoI and SalI and inserted into the XhoI site 5' of the *neo* gene of pJNS2. Thus, the final targeting construct is identical to the corresponding region in the mouse genome, except that a 0.3 kb BglII–BamHI fragment that encompasses the 3' end of intron III and the 5' end of exon IV has been replaced by the positively selectable *neo* gene in pJNS2.

ES Cell Culture and Electroporation

The ES cell lines D3 (Doetschman et al., 1987) and E14TG2a (Hooper et al., 1987) were cultured as previously described (Koller and Smithies, 1989). Electroporation was carried out in culture medium containing 3 nM of targeting plasmid (linearized with NotI), using conditions described previously, except that a 1 s discharge from a 150–250 mF capacitor charged to 250–400 V was used. Transfected colonies were selected by growth in G418 and enriched for targeted colonies by the addition of gancyclovir (a gift from Syntex, Palo Alto, California) to the growth medium. Surviving colonies were expanded to a level sufficient for PCR and Southern blot analysis.

Southern Blot and PCR Analyses

For PCR screening, primers were chosen in the pJNS2 polylinker (bases 2–35) and 0.15 kb 3' from exon V (GCTCTAGCAACAGAGG-CAGATTAC). The length of the expected product was 1.75 kb for cases of homologous recombination and no amplification in cases of random integration. Southern blot analyses were performed after digestion with EcoRI using standard conditions. Blots were hybridized with either a 0.35 kb HindIII–BamHI fragment that is located 0.15 kb downstream of exon V or a 0.6 kb XhoI–PstI fragment of the *neo* expression cassette.

Animals

Chimeric animals were generated as described and bred to either B6D2 or C57BL/6 mice (Koller and Smithies, 1989). Offspring carrying the targeted α gene were identified by Southern analysis of tail DNA prepared as described (Miller et al., 1988). All the experiments were performed on 4- to 8-week-old mice. Each individual experiment was performed on (+/+) and (-/-) littermates.

Histological Analysis

Mast cells were stained with acidic toluidine blue (pH 1.5), Giemsa, or Alcian blue–safranin. The number of mast cell per square millimeter was determined. For each tissue, 25 microscopic fields were counted on five nonserial sections.

Flow Cytometry

BMMCs were isolated from 4- to 8-week-old mice according to Rottem et al. (1992). Samples (2×10^5 to 5×10^5 cells) of BMMCs, thymocytes, or splenocytes were analyzed by flow cytometry. FcεRI was detected using biotinylated mouse monoclonal anti-DNP IgE (20 μg/ml) and streptavidin–phycoerythrin (10 μg/ml) (Molecular Probes Incorporated) after preincubation with a rat anti-murine FcγRII/III (2.4G2) monoclonal antibody (10 μg/ml). Unstained cells, cells stained with streptavidin–phycoerythrin alone, or cells preincubated with a 10-fold excess of unlabeled IgE were used as controls. Interaction of IgE with FcεRI/CD23 was assessed by inhibition of binding of biotinylated IgE with a rat anti-murine CD23 monoclonal antibody (B3B4) (10 μg/ml) (Pharmingen) after preincubation with 2.4G2 (10 μg/ml). Presence of FcγRII and FcγRIII was detected using 2.4G2–fluorescein isothiocyanate (FITC) (10 μg/ml). Binding of IgE–antigen ICs and of oligomeric IgG to FcγRII/III was performed as described previously (Takizawa et al., 1992). Mast cells were identified using rabbit polyclonal anti-murine c-Kit receptor antibody (gift from Dr. P. Bessmer, Memorial Sloan-Kettering Cancer Center, New York) and goat F(ab')₂ anti-rabbit IgG–FITC (10 μg/ml) (Jackson ImmunoResearch). B cells were identified by detection of surface immunoglobulins using goat anti-mouse immunoglobulin–FITC (10 μg/ml) (Pharmingen).

Serotonin and Histamine Release Assay

BMMCs were incubated overnight in the presence of 1 mCi/ml of 5-[1,2-³H(N)]-hydroxytryptamine binoxalate ([³H]serotonin) (DuPont),

then washed twice, and resuspended for triggering at a density of 10^6 cells/ml as described previously (Takizawa et al., 1992). Radioactivity was determined separately in the pellet and in the supernatant. Percent release was calculated by dividing counts per minute in the supernatant by total counts per minute. Histamine assay was performed according to Rottem et al. (1992).

Serum Clearance of IgE

IgE was iodinated using chloramine T to a specific activity of 4×10^5 cpm/ μ g. [^{125} I]IgE (20 ml or 1 ml) in 200 μ l of phosphate-buffered saline (PBS) with 0.1% bovine serum albumin were injected i.v. into two (+/+) and two (-/-) mice. Blood was collected in heparinized tubes at various intervals of time. To separate the [^{125} I]IgE from free [^{125} I]iodine or degraded products, [^{125} I]IgE in blood samples (50 μ l) was immunoprecipitated with rabbit anti-murine IgE antibodies bound to protein A-sepharose resin (Pharmacia). After centrifugation for 5 min at $1500 \times g$, radioactivity of the pellet was counted. Animals were sacrificed after 24 hr; organs (ear, skin, tongue, liver, spleen, stomach, kidney, and trachea with thyroid) were taken and weighed; and radioactivity was counted.

Passive Cutaneous Anaphylaxis

Passive cutaneous anaphylaxis experiments were performed according to Wershil et al. (1987). In brief, animals were injected intradermally in their right ear with 0.1 μ g of either murine anti-DNP IgE or anti-DNP IgG in 50 μ l of PBS with 0.1% bovine serum albumin. The left ear was injected with PBS-bovine serum albumin as control. After 20 hr, animals were injected in the tail vein with 200 μ l of a solution containing 3×10^6 cpm of [^{125} I]-labeled guinea pig fibrinogen and 1 mg/ml of human fibrinogen (Sigma) in saline. Animals were receiving 0.1 mg of DNP₃₀₋₄₀-HSA i.v. after 30 min and were killed 1.5 hr later. Ears were amputated, weighed, and counted for radioactivity, and the total and urea-insoluble fibrin were determined as described (Wershil et al., 1987).

Systemic Anaphylaxis

Systemic anaphylaxis experiments were performed according to Ando et al. (1993). Animals received in the tail vein 20 μ g of either murine anti-DNP IgE or anti-DNP IgG or 400 μ g of rabbit anti-TNP polyclonal antibody (Pharmingen). After 22–24 hr, mice were i.v. injected with 1 mg of DNP₃₀₋₄₀-HSA in 200 μ l of PBS containing 0.5% Evans blue dye. Control mice were injected with dye alone. Temperature was monitored using a rectal probe before and at various intervals after antigen injection.

Acknowledgments

This work was supported in part by research grant R01 DK 46003-01 with equivalent cofunding by the National Heart, Lung, and Blood Institute. D. D. was supported in part by the Belgian Fund for Scientific Research (FNRS). Both D. D. and V. F. were supported in part by a North Atlantic Treaty Organization fellowship. The authors are grateful to Drs. A. Scharenberg, R. Numerof, and P. Roche for critical reading of the manuscript and to Drs. M. Adamczewski, B. K. Wershil, and S. J. Galli for helpful advice. We thank S. Germana for help in the preparation of the construct; A. Latour for work with (ES) feeder layers; and D. Garmon, B. Young, and N. Elder for mouse husbanding.

Received August 26, 1993; revised September 21, 1993.

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Note Added in Proof

The paper cited as Maurer et al., submitted, is now in press: Maurer, D., Fiebiger, E., Reininger, B., Wolff-Winiski, B., Jouvin, M.-H., Kilgus, O., Kinet, J.-P., and Stingl, G. (1993). Expression of functional high-affinity immunoglobulin E receptors (FcεRI) on monocytes of atopic individuals. *J. Exp. Med.*, in press.