Anaphylaxis Mediated Through a Humanized High Affinity IgE Receptor[1]

David Dombrowicz,* Anna T. Brini,† Véronique Flamand,* Elizabeth Hicks,‡ John N. Snouwaert,‡ Jean-Pierre Kinet,§ and Beverly H. Koller[2‡]

Mast cells and basophils, which are activated by IgE and allergens through the high affinity IgE receptor (FcεRI), play a prominent role in anaphylaxis in the mouse. Mice deficient in this receptor become resistant to passive anaphylaxis. As a first step in developing an in vivo model that more closely mimics the IgE-mediated responses in man, we used a combination of transgenic and embryonic stem cell technology to generate a mouse line in which the murine FcεRI α-chain has been replaced with its human homologue. We demonstrate here that these mice express a tetrameric high affinity IgE receptor, in which the human α-chain associates with the murine β- and γ-chains, and that upon triggering with relevant Ag, this receptor mediates the initiation of the expected intracellular events. In addition, we show that the human α-chain restores an anaphylactic response to the nonresponsive α-deficient parental mouse line. This "humanized" mouse represents a potentially important model system, not only for studying the role of IgE in human immune responses, but also for testing potential therapeutic reagents that can interfere with responses mediated through the human FcεRI receptor. *The Journal of Immunology,* **1996, 157: 1645–1651.**

Attempts to understand the workings of the human immune system have led to the development of a wide variety of experimental systems. These include systems in which the interactions of isolated components of the immune system can be studied in vitro as well as animal models that allow immune responses to be studied in the complex environment where they normally occur. Of the animal models that have been developed for the study of immune responses in vivo, the mouse is the species that has been the most widely used. However, despite the fact that studies using mice have provided valuable insights into the workings of the human immune system, the application of information generated by such studies to the treatment of human disease has often been limited by the fact that murine and human immune responses differ from one another in many important ways. To increase the usefulness of the mouse as a model system for understanding human immune responses, it would therefore be desirable to create mice in which the immune system more closely resembles that of humans. This has been accomplished at the cellular level by introducing human hemopoietic stem cells into mice such as the severe combined immunodeficiency mouse, which carries a mutation that impairs the development of the endogenous hemopoietic system (1). Another approach would alter mice at the genetic level, replacing genes that encode important immune functions with their human homologues (2). To develop better models for the study of anaphylaxis and allergic responses in vivo, we have used this second approach to create mice in which the endogenous gene encoding the α-subunit of the high affinity IgE receptor, FcεRI,[3] has been inactivated and replaced with its human homologue.

FcεRI belongs to the Fc receptor superfamily (3, 4). It has a tetrameric structure composed of one α-, one β-, and two γ-subunits. The α-subunit directly binds IgE (5), while the β- and γ-subunits appear to function primarily in signal transduction (6). FcεRI receptors on mast cells and basophils undergo aggregation upon binding IgE and multivalent Ags, leading to the release of mediators of allergic reactions. Mast cells in both humans and mice express receptors in addition to the high affinity IgE receptor that bind IgG and IgE (7, 8). In humans, FcεRI is also present on Langerhans cells (9, 10), on monocytes from atopic individuals (11), and on eosinophils in some pathologic states (12). Transfection experiments have shown that the presence of the β-subunit is necessary for surface expression of rodent receptors, while the γ-subunit alone has been found to be sufficient to allow surface expression of the human α-subunit (13).

The central role of FcεRI in mediating anaphylaxis was established by the demonstration that mice deficient in this receptor fail to undergo passive anaphylaxis (14). These mice were generated by homologous recombination in ES cells using a plasmid designed to disrupt the mouse α chain. Here we report the reconstitution of the anaphylactic response in these mice through the introduction into their germ line of a transgene encoding the human (h) FcεRI α-chain.

*Molecular Allergy and Immunology Section, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892; †Department of Pharmacology, Chemotherapy, and Medical Toxicology, University of Milan, Milan, Italy; ‡Department of Medicine, University of North Carolina, Chapel Hill, NC 27599; and §Department of Pathology, Harvard Medical School, Beth Israel Hospital, Boston, MA 02115

Received for publication December 7, 1995. Accepted for publication May 23, 1996.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] This work was supported by National Institutes of Health Grant R01DK46003-01, with equivalent cofunding by the National Heart, Lung, and Blood Institute, and Grant DK38103 (to B.H.K.). D.D. is supported in part by a fellowship from the FNRS (Belgium). A.T.B. is supported in part by grants from NATO (CRG 940134) and Telethon (Italy; D14).

[2] Address correspondence and reprint requests to Dr. Beverly Koller, Department of Medicine, 724 Burnett-Womack Building CB-7020, University of North Carolina, Chapel Hill, NC 27599.

[3] Abbreviations used in this paper: FcεRI, high affinity IgE receptor; ES, embryonic stem; h, human; m, mouse; HPRT, hypoxanthine phosphate ribosyltransferase; BMMC, bone marrow-derived mast cells; NIP, 4-hydroxy-3-nitrophenylacetyl; PVDF, polyvinylidene difluoride; HSA, human serum albumin.

0022-1767/96/$02.00

Materials and Methods

Isolation and characterization of the human α gene

A 9.5-kb *Bam*HI fragment containing the hFcεRIα gene was isolated from a human leukocyte genomic library generated with the EMBL3 λ phage vector (Clontech, Palo Alto, CA) using the full-length hFcεRIα cDNA as a probe (15).

Transgenic animals

Five micrograms of the 9.5-kb fragment containing the hFcεRIα gene and 5 μg of the plasmid HPRT50 (16), a plasmid containing the *hprt* minigene, were introduced by calcium phosphate precipitation into the ES cell line ES65–154 (14) essentially as described previously (17).

ES cell colonies were visualized 10 days later after selection in hypoxanthine-aminopterin-thymidine-medium, and individual colonies were picked and expanded. DNA was prepared from 12 of these colonies. Analysis by Southern blot, using the full-length hFcεRIα cDNA as a probe, showed that nine of these had integrated the transgene. ES cells from two unique clones were introduced into 3.5-day-old C57Bl/6 blastocysts and implanted into foster mothers. Chimeras were generated from both of these lines. Female chimeras were used for the analysis described below. Male chimeras, which are in general more likely to transmit the ES cell genome than their female siblings, were bred to female FcεRIα −/− mice to obtain mice that were homozygous for the mutant murine FcεRIα gene and that also carried the transgene encoding hFcεRIα.

Bone marrow-derived mast cells (BMMC)

BMMC were obtained as previously described (18). Briefly, bone marrow was aseptically obtained from 6-wk-old animals and placed in culture for 3 wk in the presence of IL-3-conditioned medium. Nonadherent cells were transferred weekly into fresh medium.

Antibodies and FACS staining

Murine anti-DNP IgE (clone SPE-7) was obtained from Sigma Chemical Co. (St. Louis, MO). TANE, a chimeric IgE molecule (hIgE-Fc, mouse anti-NIP IgE-Fab) was obtained from Serotec (19). TANE will be referred to as hIgE throughout this article.

The 15-1 anti-hFcεRIα mAb has been described previously (10). FACS staining was performed for 30 to 60 min at 4°C in 100 μl of PBS containing 0.1% BSA and 0.05% sodium azide at 5×10^6 cells/ml. All murine cells were preincubated before staining with hybridoma supernatant from 2.4G2 Ab-producing cells (20) to block IgE binding to FcγRII/RIII. Concentrations used for staining were 10 μg/ml for hIgE-biotin and 1/2500 for streptavidin-phycoerythrin (PharMingen, San Diego, CA). The specificity of binding to hFcεRIα was checked by preincubation with 150 μg/ml unlabeled 15-1. To quantify the expression of the mouse receptor on BMMCs, cells were preincubated with 15-1 and then stained with DNP-specific biotinylated mouse IgE and 1/2500 diluted streptavidin-phycoerythrin. Samples were analyzed on a FACScan cytofluorimeter (Becton Dickinson, Mountain View, CA).

Western blot

BMMCs (1.5×10^7) from wild-type and transgenic animals were loaded at 4°C for 3 h with 10 μg/ml 15-1 at 5×10^6 cells/ml and then washed three times with PBS. Cells were lysed at 5×10^7cells/ml in 1% digitonin, 150 mM NaCl, 50 mM HEPES (pH 7.4), 10 μg/ml aprotinin, 10 μg/ml pepstatin, 5 μg/ml leupeptin (ICN Biochemicals, Costa Mesa, CA) on ice for 30 min. The lysate was then centrifuged for 20 min at 4°C. The supernatant was removed and immunoprecipitated for 2 h at 4°C with 40 μl of GammaBind Plus Sepharose beads (Pharmacia, Piscataway, NJ) and then for an additional 2 h with 40 μl of beads coated with 10 μg of 15-1. Samples were loaded on 14% SDS-polyacrylamide Tris-glycine gel (Novex Experimental Technologies). After electrotransfer, PVDF membrane (Millipore Corp., Bedford, MA) was probed with a mAb against rat FcεRIβ (JRK) (21) and anti-murine FcεRIγ antiserum (22). Revelation was performed using anti-mouse and anti-rabbit horseradish peroxidase-conjugated Abs (Amersham Corp., Arlington Heights, IL) and the ECL kit (Amersham) according to the manufacturer's instructions. Lysates from BMMCs, thymocytes, and splenocytes were immunoprecipitated with the 15-1 Ab, and the immunoprecipitate was fractionated under nonreducing conditions on 8% acrylamide gels, electrotransferred to a PVDF membrane, and probed with a polyclonal Ab specific for the hFcεRI α-chain. Revelation was performed using anti-mouse and anti-rabbit HRP-conjugated Abs (Amersham) and the ECL kit (Amersham) according to the manufacturer's instructions.

Calcium flux measurement

BMMC from either transgenic or wild-type animals were loaded at 5×10^6 cells/ml for 45 min at 25°C with the acetoxymethylester of fura-2 (Molecular Probes, Eugene, OR; 2 μM) in a buffer, pH 7.4, containing 135 mM NaCl, 5 mM KCl, 1 mM $CaCl_2$,1 mM $MgCl_2$, 5.6 mM glucose, 10 mM HEPES, 0.1% BSA, and 2.5 mM Probenecid. Cytoplasmic free Ca^{2+} was measured on a Photon Technology International Deltascan 4000 spectrofluorimeter at 500 nm with a cell suspension alternatively excited at 340 and 380 nm. Cells were loaded first with 5 μg/ml hIgE and triggered with 100 ng/ml NIP-BSA, then with 5 μg/ml mIgE and triggered with 100 ng/ml DNP-HSA. Calcium concentrations were calculated using the published value of 2.24×10^{-7} for the K_d for fura-2 at 37°C (23).

Serotonin release assay

A serotonin release assay was performed according to Takizawa et al. (8). BMMC from chimeric mice as well as from wild-type animals were loaded overnight with 1 μCi/ml 5-[1,2-*N*-^{3}H]hydroxytryptamine binoxalate ([^{3}H]serotonin) at 1×10^6 cells/ml in complete RPMI. For triggering through the human receptor, cells were loaded with 5 μg/ml hIgE (anti-NIP) at 37°C for 2 h at 2.5×10^6 cells/ml in medium. For selective triggering through the murine receptor, cells were first loaded with 75 μg/ml nonspecific hIgE (obtained from human serum) for 1 h then with 5 μg/ml murine anti-DNP IgE for 2 additional h. The cells were washed three times with medium and incubated at 2.5×10^6 cells/ml with various concentrations of specific Ag (NIP-BSA for hIgE and DNP-HSA for mIgE). Duplicate samples were analyzed for each Ag concentration.

Passive systemic anaphylaxis

Passive systemic anaphylaxis was performed as previously described (14). Briefly, animals were injected i.v. with 200 μl of PBS containing 20 μg of a monoclonal mouse anti-mouse DNP IgE and 24 h later with 200 μl of PBS containing 1% Evans blue dye and 1 mg of DNP-HSA. Control animals received only Ag. Baseline temperature was established for each animal using a rectal probe before injection of the Ag. Temperature drops due to anaphylactic response were recorded 30 min after the injection of Ag. Animals were then killed, and edema was quantitated. Seven-millimeter ear punches were obtained from each ear, and the Evans blue dye was extracted by incubation in 1 ml of formamide at 55°C for 48 h and was quantitated by measuring the absorbance of formamide at 610 nm with a spectrophotometer (24). Significance of results was determined using a two-sample *t* test.

Results

Generation of mice expressing hFcεRIα

The initial transgene construct with which we attempted to obtain expression of hFcεRIα in mice was based on the plasmid pCDL-SRα (25). The transgene in this construct consisted of a hFcεRIα cDNA driven by a SV-HTLV promoter with a SV40 poly(A) signal. To allow for selection of stable integrants after electroporation into mouse ES cells, this construct was modified by the addition of a neomycin resistance gene downstream of the FcεRIα transgene to yield a plasmid designated pHuαNeo. Although the pCDL-SRα construct had been demonstrated previously to yield expression of hFcεRIα after transfection into RBL, Chinese hamster ovary, and NIH3T3 cells, the pHuαNeo did not yield detectable expression of the hFcεRIα transgene in mice after integration into the murine genome. One possible reason for this apparent lack of expression was the absence in the cDNA-based construct of uncharacterized *cis*-activating elements in the 5′ noncoding region or introns of the endogenous FcεRIα gene. To circumvent this problem, we created a new construct using genomic rather than cDNA sequences. We isolated a 9.5-kb *Bam*HI fragment from a human genomic library, using a full-length human cDNA as a probe (Fig. 1). This clone encompasses the entire coding sequence of the gene as well as 2.9 kb of upstream sequence, which extends 1.6 kb upstream from the previously published sequence (26).

The hFcεRIα gene was introduced by Ca_2PO_4 precipitation into the mouse ES cell line, E14TG2a-165. To allow for enrichment of ES cell clones that had stably integrated the transgene, an *hprt*

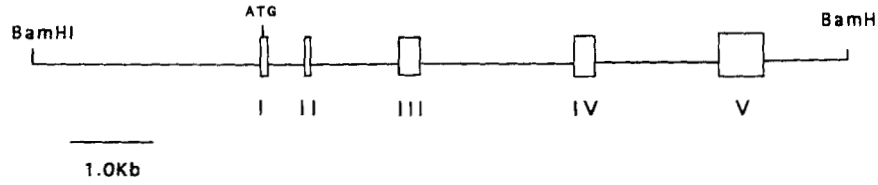

FIGURE 1. Genomic organization of the hFcεRIα gene and its 5′ regulatory regions. Exons are represented by open boxes.

minigene was co-electroporated with the DNA fragment. Hypoxanthine aminopterin thymidine-resistant ES cell clones were screened for the presence of the hFcεRIα gene by Southern blot analysis using a human cDNA probe. Examination of 12 clones identified 9 that contained at least one copy of the transgene. Because the ES cell line into which the transgene was introduced already carried a targeted null mutation in one of its endogenous FcεRIα alleles, the genotype of those cells that had integrated the transgene was hFcεRIα +, mFcεRIα +/−. Two of the ES cell lines carrying the transgene were introduced into 3.5-day-old embryos to produce chimeric mice.

Expression of hFcεRIα on mouse mast cells

Expression of the hFcεRIα transgene was first verified by FACS analysis of BMMC from female chimeras (data not shown). Results using chimeras were later verified by FACS analysis of mast cells from the offspring of chimeras (Fig. 2*A*). BMMCs from mice that were genotyped as hFcεRIα +, mFcεRI −/− were expanded in culture in the presence of IL-3. Surface expression of hFcεRIα was detected by incubating the expanded mast cells with biotinylated hIgE followed by streptavidin-phycoerythrin. FACS analysis revealed high levels of surface expression of hFcεRIα in the transgenic mice, but not in control littermates. The specificity of this binding was confirmed by the fact that it could be inhibited by preincubation of mast cells with the 15-1 Ab, which is specific for hFcεRIα.

To estimate the level of expression of the humanized receptor relative to that of the endogenous gene, BMMCs from mice carrying the transgene and one copy of the endogenous gene were stained with biotinylated hIgE or with mouse IgE after blocking the binding to the human receptor with 15-1 Ab. The staining intensities of the two different IgE preparations had been calibrated previously using Chinese hamster ovary cells transfected with the hFcεRI α-, β-, and γ-chains to ensure that signals with the two reagents were comparable and, thus, that differences in staining intensity would reflect differences in expression of the two receptors. In mice carrying the transgene, the humanized receptor was found to be expressed on BMMCs at levels approximately two- to fivefold higher than those normally observed for the endogenous receptor in nontransgenic controls.

In FcεRIα +/− mice carrying the transgene, the level of the endogenous mouse receptor at the cell surface was lower than that in FcεRIα +/− controls that lacked the transgene. This was true for mice derived from both of the original ES cell lines carrying the transgene. We hypothesize that this decrease is caused by competition of the endogenous α-chain with the human α-chain for limited numbers of β- and γ-chains.

To determine whether the 5′ sequences included in the transgene were sufficient to impart an expression pattern similar to that of the endogenous α-chain, we examined RNA isolated from various tissues for message derived from the transgene. No expression of the transgene was seen in RNA prepared from kidney, liver, testes, spleen, or thymus (data not shown). In addition, Western blot analysis using Abs specific for the hFcεRI α-chain was conducted on digitonin-solubilized protein preparations from transgenic mice. While this approach revealed no expression of the humanized receptor in thymocytes or splenocytes, it provided clear evidence for expression of the receptor in BMMCs (Fig. 3*A*).

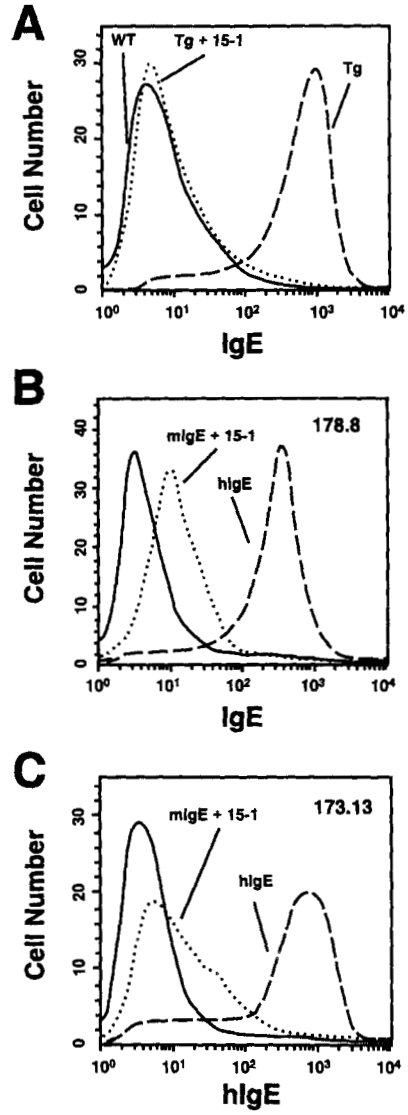

FIGURE 2. Surface expression of the hFcεRIα on BMMCs from human α transgenic mice and comparison to endogenous levels of receptor. *A*, BMMCs were prepared from mice homozygous for the mutant mouse allele and carrying the transgene. Cells were labeled with biotinylated hIgE and streptavidin-phycoerythrin. Transgenic mouse (dashed line), wild-type mouse (solid line), and transgenic mouse treated with 15-1 Ab before staining with hIgE (dotted line) are indicated. *B* and *C*, BMMCs were prepared from each of the two transgenic lines. Mice were also heterozygous for the targeted endogenous FcεRIα gene. Cells were labeled with biotinylated hIgE and streptavidin-phycoerythrin (dashed line), with biotinylated mouse IgE streptavidin-phycoerythrin after blocking the humanized receptor with 15-1 Ab (dotted line), or with streptavidin alone (solid line).

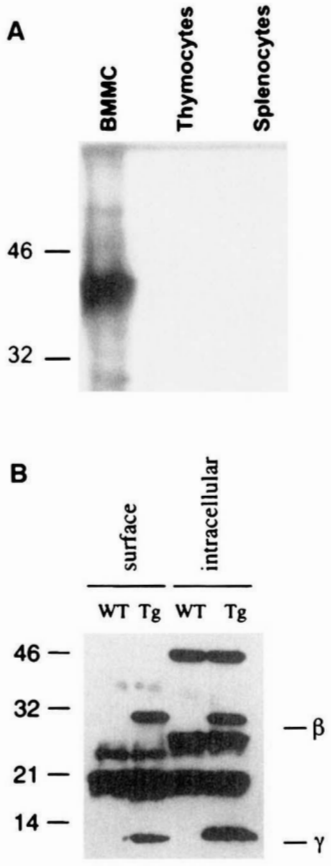

FIGURE 3. Expression and structure of the humanized FcεRI at the surface and in the cytoplasm of BMMC from transgenic mice. Digitonin-solubilized protein extracts from BMMC, thymocytes, and splenocytes were immunoprecipitated with the 15-1 mAb, and immunoprecipitated material was loaded onto an 8% acrylamide gel under nonreducing conditions. Western analysis was carried out with polyclonal antiserum (997) directed against the hFcεrIα chain. *A* and *B*, BMMC from wild-type and transgenic mice were loaded with 15-1 monoclonal anti-hFcεRIα Ab, washed, and lysed with 1% digitonin, then sequentially immunoprecipitated with protein G beads (surface) and beads loaded with 15-1 Ab (intracellular). Samples were run on a 14% gel and transferred to a PVDF membrane. The upper part of the membrane was probed with an anti-rat FcεRIβ, and the lower part was probed with an anti-mouse FcεRIγ. The molecular mass is indicated in kilodaltons.

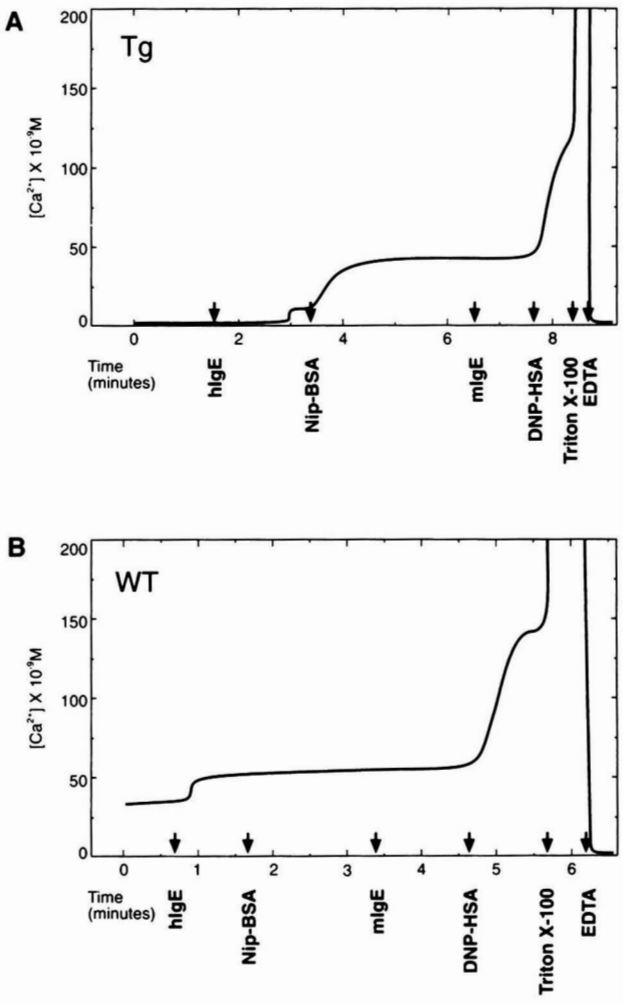

FIGURE 4. Functionality of the humanized FcεRI on BMMC from transgenic mice. Calcium flux upon triggering of the humanized and murine FcεRI from transgenic (*A*) and wild-type (*B*) mice. BMMC were loaded with fura-2 and sequentially loaded and triggered with hIgE and NIP-BSA, respectively, and then with mIgE and DNP-HSA, respectively. Cells were lysed with 1% Triton X-100 and Ca^{2+} chelated with EDTA.

To determine whether the hFcεRIα protein in our transgenic mice had formed a complex with the mFcεRI β- and γ-chains, mast cells were incubated with the 15-1 Ab. After washing to remove unbound Ab, cells were lysed, and surface-expressed Ab-receptor complexes were precipitated with protein G-Sepharose beads. The precipitated proteins were analyzed by PAGE followed by Western blot analysis with Ab specific for the mFcεRI β- and γ-chains (Fig. 3). This analysis showed that the human α-chain had indeed associated with the mouse β- and γ-chains. The presence of similar complexes in the cytoplasm of mast cells was demonstrated by precipitation of additional receptors with 15-1 bound to protein G beads from the cell lysate after the initial treatment.

Activation of mast cells through the humanized receptor

Previous studies have elucidated the chain of events by which mast cells are activated by interaction of IgE with the FcεRI receptor. After IgE has bound to the α-chain of the receptor, binding of Ag to IgE leads to receptor aggregation. This aggregation is followed by activation of tyrosine kinases and subsequent phosphorylation of the FcεRI receptor subunits. One indicator of subsequent mast cell degranulation is the release of serotonin. To determine whether this cascade of events could be triggered through the humanized FcεRI receptor on mast cells from transgenic mice, we examined increased intracellular calcium and serotonin release as indexes of mast cell activation. These experiments were performed on BMMC from chimeric animals, in which tissues were composed of a mixture of blastocyst- and ES cell-derived cells. In such chimeras, cells derived from the blastocyst are mFcεRIα +/+, while those derived from the injected ES cells are mFcεRIα +/− hFcεRIα +. Thus, it is expected that, regardless of the functionality of the humanized FcεRI receptor, IgE-mediated responses in mast cells derived from these animals should be evoked by binding of murine IgE to the mFcεRI.

To test the ability of the humanized FcεRI to trigger an intracellular calcium influx, mast cells grown from chimeras and wild-type animals were expanded in culture in the presence of IL-3 and then loaded with hIgE specific for NIP. As expected, this treatment by itself did not result in a detectable rise in intracellular Ca^{2+}.

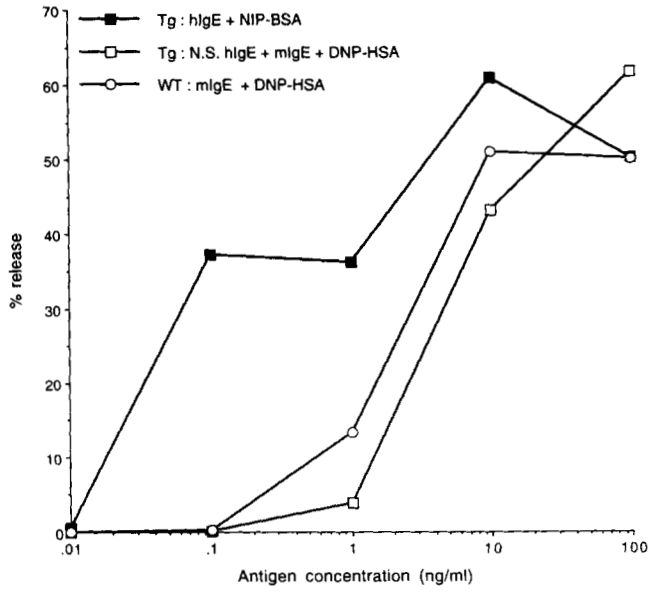

FIGURE 5. Serotonin release induced upon triggering of the humanized and murine FcεRI from transgenic and wild-type mice. Cells were loaded overnight with [^{3}H]serotonin. Triggering of the mast cells was performed as indicated in the figure.

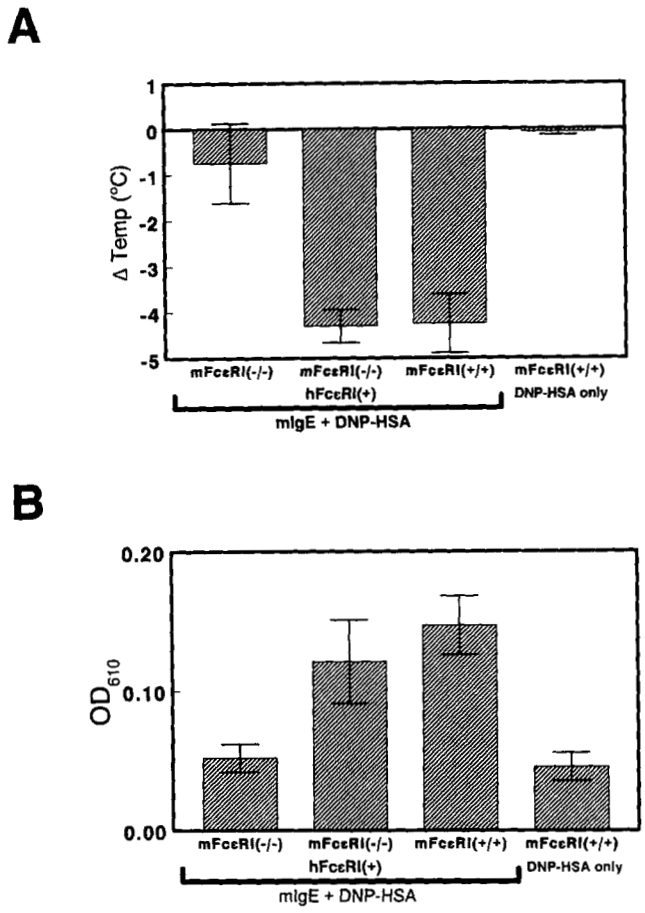

FIGURE 6. IgE-mediated systemic anaphylaxis in mice expressing a humanized FcεRI receptor. *A*, Changes in rectal temperatures of hFcεRIα + mFcεRIα −/− (n = 6), FcεRIα +/+ (n = 4), and FcεRIα −/− (n = 5) mice during IgE-induced systemic anaphylaxis. Differences in temperature taken just before the injection of Ag (1 mg of DNP_{30-40}-HSA) and 30 min thereafter were recorded for each animal. One group of wild-type animals received only Ag, all other animals had received 20 μg of murine anti-DNP IgE 24 h before the initiation of the experiment. Error bars represent the SEM. *B*, Changes in vascular permeability in the hFcεRIα + mFcεRIα −/− (n = 4), FcεRIα +/+ (n = 7), and FcεRIα −/− (n = 5) mice during IgE-induced systemic anaphylaxis. A 7-mm ear biopsy was taken from all animals 30 min after the injection of 1 mg of DNP_{30-40}-HSA in 0.5% Evans blue dye. All animals, with the exception of the wild-type group indicated, had received 20 μg of anti-DNP IgE 24 h before the initiation of the experiment. Error bars represent the SEM.

However, addition of NIP-BSA to the hIgE-loaded mast cells resulted in an increase in free cytoplasmic Ca^{2+} in the mast cells derived from the chimeras, but not in those from the wild-type animals (Fig. 4). This same experiment was continued by the addition of mouse IgE specific to DNP followed by DNP-HSA. As expected, this treatment resulted in an increase in free cytoplasmic Ca^{2+} in BMMC from both wild-type and chimeric animals.

To assess the ability of the humanized FcεRI receptor to trigger serotonin release, mast cells from chimeric mice and wild-type controls were expanded in the presence of IL-3 and loaded with radioactive serotonin. Incubation of the cells with the hIgE specific for NIP followed by activation with the Ag resulted in the release of serotonin from the mast cells in a dose-dependent manner (Fig. 5).

We also sought to compare the level of mast cell activation achievable through humanized FcεRI with that achievable through mFcεRI. As mouse IgE can bind to the human receptor, it was necessary to block humanized FcεRI by the addition of an excess of polyclonal hIgE before incubating cells with a monoclonal mouse IgE specific for DNP. Subsequent addition of DNP-HSA resulted in the release of serotonin from the mast cells. Mast cell activation through the human receptor was achieved at far lower levels of Ag for several reasons. First, the high level of chimerism in these animals, as determined by coat color, suggested that cells carrying the human receptor were likely to greatly outnumber those derived from the host blastocyst. In addition, as discussed above, the presence of the human receptor significantly reduced the level of the mouse receptor relative to that normally seen in BMMCs (Fig. 2, *B* and *C*). Finally, differences in valance between the NIP and DNP Ags could contribute to differences in the concentrations of these molecules required for triggering the mast cells.

Restoration of anaphylaxis

Allergy-like reactions are some of the most important biologic responses mediated through the FcεRI receptor. To determine whether the humanized FcεRI receptor expressed in our transgenic mice could trigger such reactions, we examined passive systemic anaphylaxis in mice with three different genotypes: mFcεRIα −/−, hFcεRIα + mFcεRIα −/−, and mFcεRIα +/+. In wild-type mice, passive systemic anaphylaxis is typically characterized by a number of physiologic changes, including increased vascular permeability, generalized fluid extravasation, and profound shock, exhibited by a drop in blood pressure and body temperature (27).

To elicit the anaphylactic response, mice were injected i.v. with a monoclonal IgE Ab specific for DNP. Twenty-four hours later, the DNP Ag was administered. To allow quantitation of fluid extravasation, Evans blue dye was administered with the Ag. This dye binds to serum proteins and remains largely confined to the circulatory system before the change in permeability of the vessels following Ag challenge. Extravasation of the dye during the anaphylactic response allows the intensity of the response to be visualized and quantitated. Control mFcεRIα +/+ animals received only the Ag and Evans blue dye.

The anaphylactic responses in the three groups of animals were quantitated in two ways. First, the rectal temperatures of the animals were monitored during the responses (Fig. 6*A*), and second, the increase in vascular permeability was monitored by measuring Evans blue dye in ear tissue (Fig. 6*B*). As expected, a significant drop in temperature was seen in the wild-type mice relative to that in controls treated with DNP only ($p = 0.003$). This drop was virtually eliminated in mice homozygous for the null FcεRIα allele, which did not differ significantly in their responses from control animals ($p = 0.48$). However, the temperature drop observed in hFcεRIα + mFcεRIα −/− mice was significant ($p = 0.00004$) and did not differ significantly from that observed in wild-type controls ($p = 0.93$). Although changes in vascular permeability observed in FcεRIα −/− mice did not differ significantly from those in controls ($p = 0.66$), mice expressing hFcεRIα did display a significant increase in vascular permeability ($p = 0.05$). The change in vascular permeability observed in mice expressing hFcεRIα was not significantly different from that in wild-type mice ($p = 0.49$).

Discussion

Mice expressing the humanized IgE FcεRI receptor will provide an important tool for investigating a number of aspects of the biology of the FcεRI receptor. In addition to allowing investigation of the molecular mechanisms underlying the tissue-specific distribution of the FcεRI receptor, they should provide a powerful means of assessing the effectiveness of therapeutic agents designed to intervene in IgE-mediated allergic reactions.

To generate mice expressing a humanized FcεRI receptor, we used an approach that combined gene targeting through homologous recombination with transgene expression. The production of FcεRIα-deficient mice by targeted inactivation of the FcεRIα gene has been described previously (14). To obtain expression of a humanized FcεRI receptor in these mice, we first transfected a transgene that encoded the human α-chain into an ES cell line in which one of the endogenous FcεRIα alleles had been previously inactivated by gene targeting. For this purpose we used the same cell line that had originally been used to produce the FcεIRα-deficient mice. By introducing the transgene into the mouse germ line via ES cells, we were able to examine the expression pattern of the transgene in female chimeras, thus avoiding the large amount of breeding that would have been required if we had introduced the transgene by injection into the pronucleus of single cell mouse embryos. The testing of these chimeras allowed us to quickly determine that the cDNA constructs in which the α gene was driven by the HTLV promoter would not yield animals that expressed the protein on mast cells. The relatively small size of the α-chain gene made it possible to use a genomic fragment containing the entire gene, which did yield appropriate expression of the α-chain on the surface of mast cells. In addition, the lack of detectable expression in other cell types, such as neutrophils, splenocytes, and thymocytes, supported the contention that the transgene was able to direct expression of the human α-chain in a tissue-specific manner.

The human α-chain has been shown to differ from that of the mouse in that it can be expressed at the cell surface in Langerhans cells and also in that its expression in monocytes does not require expression of the β-chain. Future examination of the transgenic mice for expression of the humanized FcεRI receptor on Langerhans cells and monocytes should provide more information about the molecular mechanisms underlying differences in expression pattern between mice and humans. If the expression pattern of the humanized receptor mirrors that in humans, the comparison of inflammatory responses in wild-type mice with those in animals expressing the humanized receptor should give an indication of the importance of Langerhans cells and monocytes in hIgE-mediated responses.

For mice expressing the humanized FcεRI receptor to provide a useful model of responses mediated through the hFcεRI receptor, it was first necessary to show not only that the humanized receptor was expressed at the surface and could bind IgE, but also that it could carry out the signal transduction required for normal IgE-mediated responses. We first demonstrated coimmunoprecipitation of the endogenous FcεRIβ and γ-chains with an anti-hFcεRIα Ab, thus establishing that the human α-chain was able to form receptor complexes with the murine β- and γ-chains. We then showed that the humanized receptor is as efficient as the endogenous murine receptor in initiating signal transduction, as assessed by the release of intracellular Ca^{2+} and subsequent release of serotonin. These data also provide evidence that the human α-chain is able to interact with the murine β- and γ-chains, as both chains are required for efficient signal transduction.

We have also shown that the humanized FcεRI receptor is able to mediate an in vivo anaphylactic response, since the presence of the human transgene restored this response to our FcεRIα-deficient mice. This result indicates that the transgene is expressed in those cells that normally mediate anaphylaxis. In addition, the ability of mice carrying the humanized receptor to undergo anaphylaxis upon binding of hIgE indicates that these animals will provide an important test system for the design of compounds that interfere with the binding of IgE to the hFcεRI receptor.

Acknowledgments

We thank E. Cram, T. Nguyen, K. Bertha, M. Key, and T. Mason for assistance with animal husbandry; B. Garges for assistance with mouse tail biopsies; and J. Goulet for helpful discussions.

References

1. *Bonyhadi, M. L., L. Rabin, S. Salimi, D. A. Brown, J. Kosek, J. M. McCune, and H. Kaneshima.* 1993. HIV induces thymus depletion in vivo. *Nature 363:728.*
2. Zou, Y.-R., H. Gu, and K. Rajewsky. 1993. Generation of a mouse strain that produces immunoglobulin κ chains with human constant regions. *Science 262: 1271.*
3. Jelsema, C. L., and J.-P. Kinet. 1994. The high-affinity receptor for immunoglobulin E. In *Molecular and Cellular Biology of the Allergic Response.* A. I. Levinson and Y. Paterson, eds. Marcel Dekker, New York, p. 211.
4. Ravetch, J. V., and J.-P. Kinet. 1991. Fc receptors. *Annu. Rev. Immunol. 9:457.*
5. Hakimi, J., C. Seals, J. A. Kondas, L. Pettine, W. Danho, and J. Kochan. 1990. The alpha subunit of the human IgE receptor (FcεRI) is sufficient for high affinity IgE binding. *J. Biol. Chem. 265:22079.*
6. Jouvin, M.-H., R. P. Numerof, and J.-P. Kinet. 1995. Signal transduction through the conserved motifs of the high affinity IgE receptor FcεRI. *Semin. Immunol. 7:29.*
7. Conrad, D. H. 1990. FcεRII/CD23: the low affinity receptor for IgE. *Annu. Rev. Immunol. 8:623.*
8. Takizawa, F., M. Adamczewski, and J.-P. Kinet. 1992. Identification of the low affinity receptor for immunoglobulin E on mouse mast cells and macrophages as FcγRII and FcγRIII. *J. Exp. Med. 176:469.*
9. 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 (FcεRI). *J. Exp. Med. 175:1285.*
10. Wang, B., A. Rieger, O. Kilgus, K. Ochiai, D. Maurer, D. Födinger, J.-P. Kinet, and G. Stingl. 1992. Epidermal Langerhans cells from normal human skin bind monomeric IgE via FcεRI. *J. Exp. Med. 175:1353.*
11. Maurer, D., E. Fiebiger, B. Reininger, B. Wolff-Wininski, M.-H. Jouvin, O. Kilgus, J.-P. Kinet, and G. Stingl. 1994. Expression of functional high-affinity immunoglobulin E receptors (FcεRI) on monocytes of atopic individuals. *J. Exp. Med. 179:745.*
12. Soussi Gounni, A., 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 defense against parasites. *Nature 367:183.*
13. Ra, C., M.-H. Jouvin, and J.-P. Kinet. 1989. Complete structure of the mouse mast cell receptor for IgE (FcεRI) and surface expression of chimeric receptors (rat-mouse-human) on transfected cells. *J. Biol. Chem. 264:15323.*
14. Dombrowicz, D., V. Flamand, K. K. Brigman, B. H. Koller, and J.-P. Kinet. 1993. Abolition of anaphylaxis by targeted disruption of the high affinity immunoglobulin E receptor α chain gene. *Cell 75:969.*

15. Kochan, J., L. F. Pettine, J. Hakimi, K. Kishi, and J.-P. Kinet. 1988. Isolation of the gene coding for the alpha subunit of the human high affinity IgE receptor. *Nucleic Acids Res. 85:5639.*
16. Reid, L., R. Gregg, O. Smithies, and B. H. Koller. 1990. Regulatory elements in the introns of the human *HPRT* gene are necessary for its expression in embryonic stem cells. *Proc. Natl. Acad. Sci. USA 87:4299.*
17. Mohn, A. R., and B. H. Koller. 1995. Genetic manipulation of embryonic stem cells. In *DNA Cloning–Mammalian Systems: A Practical Approach.* I. Hall, ed. Oxford University Press, Oxford, p. 142.
18. Rottem, M., S. Barbieri, J.-P. Kinet, and D. D. Metcalfe. 1992. Kinetics of the appearance of FcεRI-bearing cells in interleukin-3-dependent mouse bone marrow cultures: correlation with histamine content and mast cell maturation. *Blood 79:972.*
19. Neuberger, M. S., G. T. Williams, E. B. Mitchell, S. S. Joutial, J. G. Flanagan, and T. H. Rabbits. 1985. A hapten-specific chimaeric IgE antibody with human physiological effector function. *Nature 314:268.*
20. Unkeless, J. C. 1979. Characterization of a monoclonal antibody directed against mouse macrophage and lymphocyte Fc receptors. *J. Exp. Med. 150:580.*
21. Riviera, J., J.-P. Kinet, J. Kim, C. Pucillo, and H. Metzger. 1988. Studies with a monoclonal antibody to the β subunit of the receptor with high affinity for immunoglobulin E. *Mol. Immunol. 25:647.*
22. Orloff, D. G., C. S. Ra, S. J. Frank, R. D. Glausner, and J.-P. Kinet. 1990. A family of disulfide-linked dimers containing the ζ and η chains of the T-cell receptor and the γ chain of Fc receptors. *Nature 347:188.*
23. Grynkiewicz, G., M. Poenie, and R. Tsien. 1985. A new generation of Ca^{2+} indicators with greatly improved fluorescence properties. *J. Biol. Chem. 260: 3440.*
24. Jansco-Gabor, A., J. Szolcsanyi, and N. Jancso. 1967. A simple method for measuring the amount of azovan blue exuded into the skin in response to an inflammatory stimulus. *J. Pharm. Pharmacol. 19:486.*
25. Takebe, Y., M. Seiki, J.-I. Fujisawa, P. Hoy, K. Yokota, K.-I. Arai, M. Yoshida, and N. Arai. 1988. Srα promotor: an efficient and versatile mammalian cDNA expression system composed of the simian virus 40 early promoter and the R-U5 segment of human T-cell leukemia virus type 1 long terminal repeat. *Mol. Cell. Biol. 8:466.*
26. Pang, J., G. R. Taylor, D. G. Munroe, A. Ishaque, W.-P. Fung-Leung, C. Y. Lau, F.-Y. Liu, and L. Zhou. 1993. Characterization of the gene for the human high affinity IgE receptor (FcεRI) α-chain. *J. Immunol. 151:6166.*
27. Bochmer, B. S., and L. M. Lichtenstein. 1981. Anaphylaxis. *N. Engl. J. Med. 324:1785.*