Transgenic Mice Expressing the Human High-Affinity Immunoglobulin (Ig) E Receptor α Chain Respond to Human IgE in Mast Cell Degranulation and in Allergic Reactions

By Wai-Ping Fung-Leung,*‡ Jean De Sousa-Hitzler,‡ Armana Ishaque,‡ Lubing Zhou,*‡ Jesse Pang,*‡ Karen Ngo,*‡ Julie A. Panakos,‡ Erika Chourmouzis,‡ Fu-Tong Liu,§ and Catherine Y. Lau‡

*From the *R. W. Johnson Pharmaceutical Research Institute (La Jolla), San Diego, California 92121; ‡R. W. Johnson Pharmaceutical Research Institute (Toronto), Don Mills, Ontario M3C 1L9, Canada; and §Department of Medicine and Experimental Medicine, The Scripps Research Institute, La Jolla, California 92037*

Summary

The high-affinity receptor for immunoglobulin (Ig) E (FcεRI) on mast cells and basophils plays a key role in IgE-mediated allergies. FcεRI is composed of one α, one β, and two γ chains, which are all required for cell surface expression of FcεRI, but only the α chain is involved in the binding to IgE. FcεRI–IgE interaction is highly species specific, and rodent FcεRI does not bind human IgE. To obtain a "humanized" animal model that responds to human IgE in allergic reactions, transgenic mice expressing the human FcεRI α chain were generated. The human FcεRI α chain gene with a 1.3-kb promoter region as a transgene was found to be sufficient for mast cell–specific transcription. Cell surface expression of the human FcεRI α chain was indicated by the specific binding of human IgE to mast cells from transgenic mice in flow cytometric analyses. Expression of the transgenic FcεRI on bone marrow–derived mast cells was 4.7×10^4/cell, and the human IgE–binding affinity was $K_d = 6.4$ nM in receptor-binding studies using ^{125}I-IgE. The transgenic human FcεRI α chain was complexed with the mouse β and γ chains in immunoprecipitation studies. Cross-linking of the transgenic FcεRI with human IgE and antigens led to mast cell activation as indicated by enhanced tyrosine phosphorylation of the FcεRI β and γ chains and other cellular proteins. Mast cell degranulation in transgenic mice could be triggered by human IgE and antigens, as demonstrated by β-hexosaminidase release in vitro and passive cutaneous anaphylaxis in vivo. The results demonstrate that the human FcεRI α chain alone not only confers the specificity in human IgE binding, but also can reconstitute a functional receptor by coupling with the mouse β and γ chains to trigger mast cell activation and degranulation in a whole animal system. These transgenic mice "humanized" in IgE-mediated allergies may be valuable for development of therapeutic agents that target the binding of IgE to its receptor.

IgE antibodies, mast cells, and basophils play a pivotal role in allergic responses (1, 2). Exposure of an individual to allergens induces the production of allergen-specific IgE antibodies. Mast cells and basophils bind monomeric IgE via the high-affinity IgE receptor (FcεRI)[1] (1, 2). Subsequent exposure to allergens results in cross-linking of receptor-bound IgE on mast cells and basophils, leading to cellular activation and degranulation (1, 2). The release of a variety of potent mediators, such as histamine, proteases, and arachidonic acid metabolites, accounts for many of the symptoms in allergies (3, 4).

FcεRI has been suggested to be the key receptor in IgE-mediated mast cell exocytosis (1, 2). This notion is supported by the finding that gene-targeted mice defective in FcεRI expression are resistant to IgE-mediated anaphylaxis (5). FcεRI is a tetrameric receptor composed of one α, one β, and two disulfide-linked γ chains (1). Previous in vitro studies suggest that the binding of FcεRI to IgE is mediated by the α chain (6–8). However, cell surface expression of FcεRI requires the presence of all three subunits (9, 10). FcεRI is expressed not only on mast cells, basophils, and

[1] *Abbreviations used in this paper:* BMMC, bone marrow–derived mast cells; DNP, dinitrophenyl; FcεRI, high-affinity IgE receptor; NIP, nitro-iodo-hydroxyphenyl.

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eosinophils, but also on monocytes and Langerhans cells (2, 11–15), suggesting functions in addition to mediating degranulation.

Human IgE is highly species specific and does not cross-react with rodent receptors (6). To obtain an animal model capable of responding to human IgE in allergic reactions, we generated transgenic mice expressing the human FcεRI α chain. Our study showed that the human α chain of FcεRI alone can reconstitute a functional receptor in mice. These transgenic mice can respond to human IgE in allergic reactions.

Materials and Methods

Generation of Transgenic Mice Expressing the Human High-Affinity IgE Receptor α Chain. The human genomic clone for the FcεRI α chain that we reported previously (16) was used to construct the transgene. An 11.4-kb human genomic DNA fragment in the clone covering the entire structural gene plus a 1.3-kb promoter region and a 4.2-kb 3′ flanking region was injected into mouse embryos to generate transgenic mice. The transgene in mice was confirmed by DNA hybridization. Mouse tail DNA was digested with EcoRI and hybridized to the previously reported human FcεRI α chain cDNA (16). Transgenic founders identified by hybridization were then bred with C57BL/6J mice to obtain transgenic offspring for phenotypic and functional studies.

Detection of Transgenic mRNA. Total RNA was isolated from bone marrow–derived mast cells (BMMC) and from mouse organs according to the method described previously (16). The organs used in RNA extraction included heart, kidney, liver, spleen, stomach, and skin. mRNA encoding the human FcεRI α chain was detected by Northern blot hybridization using the previously reported human FcεRI α chain cDNA as a probe (16). mRNA for the mouse FcεRI α chain was detected by the oligonucleotide 5′-TGTCAAAGGATCCATGGACTAAGATCATG-3′, which is specific for exon 4 of the mouse FcεRI α chain gene and does not cross-react with the transgenic mRNA.

Mast Cell Cultures Derived from Bone Marrows. Mast cells from transgenic or C57BL/6J mice were prepared from bone marrow cells according to the method described by Razin et al. (17). In brief, bone marrow cells were collected from mouse femur and tibia and cultured at 3×10^5 cells/ml in Razin's medium (RPMI-1640 medium with 10% FCS, 50 μM 2-ME, 2 mM L-glutamine, 0.1 mM nonessential amino acids, 100 U/ml penicillin/streptomycin) supplemented with 20% WEHI-3B cell supernatant (American Type Culture Collection, Rockville, MD). Culture medium was changed weekly. After 3 wk in culture, the majority of the bone marrow cells differentiated into mast cells. Mast cells were identified by the presence of basophilic granules in the cytoplasm of the cells after May Grünwald/Giemsa staining.

The Binding of IgE to Mast Cells Analyzed by Flow Cytometry. BMMC from transgenic and normal C57BL/6J mice were used in flow cytometric analyses. 2×10^5 BMMC in 50 μl of staining buffer (PBS with 2% FCS, 0.1% sodium azide) were incubated for 1 h at 4°C with 2.5 μg of biotinylated human IgE (Serotec Ltd., Oxford, UK) or mouse IgG (18), followed by incubation with streptavidin–phycoerythrin (Becton Dickinson & Co., Mountain View, CA) for 20 min at 4°C. Cell-bound IgE was analyzed via flow cytometry using the FACScan® program (Becton Dickinson & Co.). Specificity of human IgE binding was studied by incubating BMMC with unbiotinylated human IgE before biotinylated human IgE, which was detected by streptavidin–phycoerythrin. The possible cross-reactivity of mouse IgE with human FcεRI α chain (6) in transgenic BMMC was eliminated by pretreating BMMC with human IgE.

FcεRI Receptor-binding Assay Using ^{125}I-IgE. Binding assays of the transgenic human FcεRI or the mouse FcεRI on BMMC were performed using ^{125}I-labeled human or mouse IgE, respectively. BMMC (4×10^6 cells/ml) from transgenic or C57BL/6J mice were incubated at 37°C for 2 h with various concentrations of ^{125}I-IgE. Cell-bound IgE was separated from free IgE by centrifugation of cells through 0.3 ml of an oil cushion (dioctyl phthalate/dibutyl phthalate, 2:3 vol/vol) at 10,000 *g* for 1 min. The dissociation constants and the receptor numbers of FcεRI on BMMC were calculated from values of the bound and free ^{125}I-IgE according to the one binding equation (GraphPad Prism; GraphPad Software Inc.).

Identification of FcεRI Subunits Complexed with the Human FcεRI α Chain. BMMC (10^7 cells) from transgenic or C57BL/6J mice were incubated overnight with 1 μg/ml biotinylated human or mouse IgE in 5 ml of culture medium. Cells were then solubilized in CHAPS buffer according to the method described by Kinet et al. (19) with some modifications. In brief, cells were lysed at 4°C in 0.2 ml of 10 mM CHAPS lysing buffer containing inhibitors for phosphatases and proteolytic enzymes (1 mM sodium orthovanadate, 12.5 mM sodium fluoride, 0.1 mM zinc chloride, 2 mM EDTA, 2 mM EGTA, 1 mM PMSF, 20 μg/ml aprotinin, and 10 μg/ml leupeptin). IgE-bound FcεRI in cell lysates was recovered by overnight incubation at 4°C with 0.1 ml packed volume of precleared avidin-conjugated agarose beads (Pierce Chemical Co., Rockford, IL). The IgE–FcεRI complex was eluted in SDS-PAGE loading buffer, separated on 12.5% SDS–polyacrylamide gels under reducing condition, and then transferred to membranes (PolyScreen; New England Nuclear, Boston, MA). The mouse FcεRI β and γ chains were detected by the rat FcεRI β chain–specific mAb and the rabbit serum specific for the mouse FcεRI γ chain (20), respectively. Antibody binding was detected with chemiluminescent reagents (ECL kit; Amersham Corp., Arlington Heights, IL).

Detection of Tyrosine Phosphorylation Triggered by Cross-linking of FcεRI. BMMC (2×10^6 cells) from transgenic or C57BL/6J mice were incubated either with 1 μg/ml of anti-nitro-iodo-hydroxyphenyl (NIP) human IgE (Serotec Ltd.) or anti-dinitrophenyl (DNP) mouse IgE (18) for 2 h at 37°C in PBS. Cells were then rinsed with PBS and incubated for 2 min at 37°C with 400 ng/ml NIP–BSA or DNP–BSA, respectively. BMMC incubated with IgE but not with the corresponding antigens were used as negative controls. Cells were lysed in SDS-PAGE loading buffer, and proteins were separated and transferred to PolyScreen membranes as mentioned above. Phosphotyrosine was detected by the phosphotyrosine-specific mouse mAb 4G10 (Upstate Biotechnology, Inc., Lake Placid, NY), and antibody binding was detected with chemiluminescent reagents from Amersham Corp.

Detection of Tyrosine Phosphorylation on FcεRI Subunits Triggered by the Cross-link of FcεRI. BMMC (10^7 cells) from transgenic or C57BL/6J mice were incubated overnight with 1 μg/ml biotinylated anti-NIP human IgE or anti-DNP mouse IgE in 5 ml of culture medium. The human or mouse IgE-coated BMMC were incubated with 1 μg/ml NIP–BSA or DNP–BSA, respectively, for 2 min at 37°C in PBS to cross-link IgE-bound FcεRI. BMMC were solubilized in CHAPS buffer as described earlier, and IgE-bound FcεRI in cell lysates was recovered by avidin-conjugated agarose beads. Proteins eluted from agarose beads were separated by reducing SDS-PAGE, transferred to PolyScreen membranes, and phosphotyrosines were detected. BMMC

treated similarly with unbiotinylated human or mouse IgE did not show any signals in phosphotyrosine detection, confirming the specificity of the immunoprecipitations.

IgE-mediated BMMC Degranulation Detected by the Release of β-Hexosaminidase. Mast cell degranulation triggered by IgE and antigens was carried out according to the methods described by Yen et al. (21). BMMC (5×10^5 cells) in 0.5 ml Tyrode's buffer were sensitized with 1 μg/ml anti-NIP human IgE or anti-DNP mouse IgE for 1 h at 37°C. After two rinses with Tyrode's buffer to remove unbound IgE, degranulation was triggered by 10-min incubation with 50 ng/ml of the antigens, DNP, or NIP conjugated to BSA. Degranulation was assayed by measuring the enzyme activity of released β-hexosaminidase using a colorimetric assay (22). Spontaneous β-hexosaminidase release was measured in samples not treated with IgE or antigen. Total β-hexosaminidase was measured after lysing cells by sonication. Percentage of β-hexosaminidase release was calculated as: (β-hexosaminidase in supernatant/total β-hexosaminidase) × 100.

Passive Cutaneous Anaphylaxis. Passive cutaneous anaphylaxis was performed in mice according to the method described by Saloga et al. (23). Transgenic mice and normal C57BL/6J mice were injected intradermally on the dorsal side with 500 ng NIP-specific human IgE in 25 μl saline, and on the ventral side with 25 μl saline for comparison. 2 h later, NIP–BSA conjugates (0.5 mg) plus 1% Evans blue in 250 μl saline i.v. was injected. Mice were killed 20 min later, and cutaneous anaphylaxis was assessed visually by the blue dye leakage from blood vessels into the skin. Mouse IgE–mediated anaphylaxis was carried out similarly using DNP-specific mouse IgE and DNP–BSA.

Results

Mast Cell–specific Transcription of the Transgene Encoding the Human FcεRI α Chain. We previously cloned the human FcεRI α chain gene and showed that the human gene is similar in genomic structure to that of the rodent (16). The human FcεRI α chain gene spans over a region of 5.9 kb and contains 5 exons. As shown in Fig. 1, an 11.4-kb DNA fragment covering the entire human α chain gene with an 1.3-kb promoter region and a 4.2-kb 3′ flanking region was used to generate transgenic mice. Transcription of the transgene in different organs and in mast cells from transgenic mice was studied by Northern blot hybridization. Mast cells used in the study were BMMC. Transgenic mRNA was detected only in BMMC, but not in the different organs from transgenic mice (Fig. 2 *A*). Tissue distribution of transgenic mRNA was identical to that transcribed by the endogenous mouse FcεRI α chain gene (Fig. 2 *B*). The data suggest that the 1.3-kb promoter region of the human FcεRI α chain gene is sufficient for regulating proper tissue-specific expression.

Cell Surface Expression of the Human FcεRI α Chain on Transgenic Mast Cells. Cell surface expression of the transgenic human FcεRI α chain on mast cells was assessed by its specific binding to human IgE. Human IgE was shown to bind to BMMC from transgenic mice, but not to that from C57BL/6J mice in flow cytometric analyses (Fig. 3, *C* and *D*). The binding of biotinylated human IgE to transgenic BMMC was completely abrogated when cells were pretreated with unbiotinylated human IgE, suggesting the specificity in the binding to human IgE (Fig. 3 *E*). ^{125}I-human IgE was also used in receptor-binding assays to determine the binding affinity of transgenic FcεRI toward human IgE. As shown in Table 1, the number of transgenic FcεRI on BMMC was 4.7×10^4/cell, and the human IgE dissociation constant was 6.4 nM. These values were comparable to those of the mousee FcεRI (1.8×10^5/cell, K_d = 2.19 nM) determined in the same experiment using mouse IgE (Table 1), and were also consistent with the values reported previously for human or mouse FcεRI on mast cells (24, 25). Taken together, the specific binding of human IgE to BMMC from transgenic mice as shown in flow cytometric analyses and in ^{125}I-IgE–binding assays confirms the cell surface expression of the human FcεRI α chain.

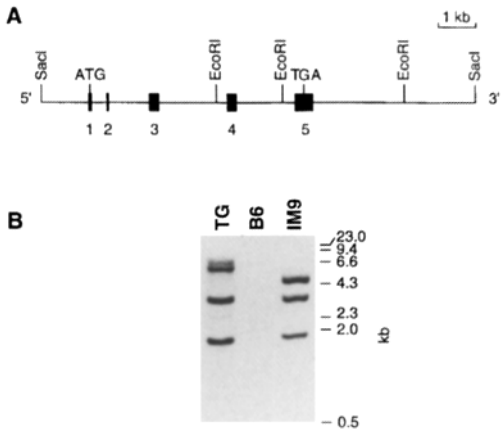

Figure 1. Expression of the human FcεRI α chain transgene. (*A*) The transgene was an 11.4-kb human genomic DNA fragment containing the entire structural gene with 5 exons (*black blocks*), a 1.3-kb promoter region, and a 4.2-kb 3′ flanking region. The location of the codons ATG and TGA, indicating the start and the end sites of translation, respectively, are shown. The transgene for embryo injection was recovered by SacI digestion. The SacI site at the 5′ end of the DNA fragment is derived from the plasmid vector. (*B*) Transgenic mice were identified by Southern blot hybridization. Tail genomic DNA samples were digested with EcoRI, and the transgene in transgenic (*TG*) mice was detected by a human FcεRI α chain cDNA probe (16). The specificity of the DNA probe was confirmed by the positive DNA bands shown in the DNA sample from a human lymphoblastic cell line (*IM9*), but not in that from the nontransgenic C57BL/6J mouse (*B6*).

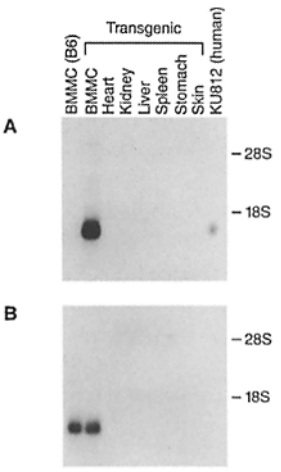

Figure 2. Tissue-specific expression of FcεRI α chain mRNA from the human transgene. (*A*) Total RNA samples from different organs (15 μg) and from BMMC (7.5 μg) of transgenic mice were detected by a human FcεRI α chain cDNA probe. (*B*) Mouse FcεRI α chain mRNA was detected by an oligonucleotide specific for the mouse gene within exon 4. RNA samples (15 μg) from the human KU812 mast cell line (41) and from BMMC of nontransgenic C57BL/6J mice (*B6*) were used as positive controls for human and mouse FcεRI α chain mRNA, respectively.

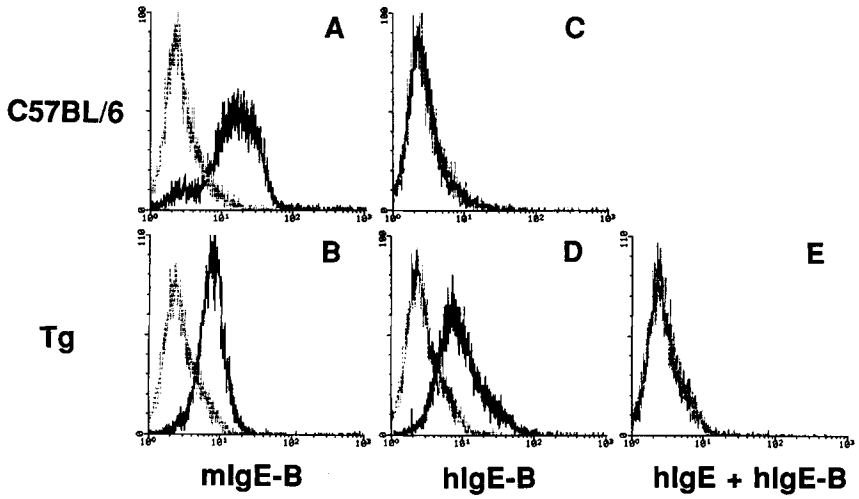

Figure 3. Cell surface expression of the transgenic human FcεRI α chain on mast cells from transgenic mice. (*A* and *B*) Histograms of cell-bound mouse IgE on BMMC from transgenic (*Tg*) mice and nontransgenic C57BL/6J mice are shown. These BMMC samples have been treated with 2.5 μg unbiotinylated human IgE for 20 min at 4°C before binding to mouse IgE. (*C* and *D*) Histograms of cell-bound human IgE on BMMC are shown. (*E*) The histogram of cell-bound human IgE on the transgenic BMMC pretreated with unbiotinylated human IgE is shown. Cells stained with streptavidin–phycoerythrin alone were used as negative controls (*gray histogram*) to compare to specific IgE binding (*black histogram*). Cell-bound IgE was analyzed by flow cytometry using the FACScan® program.

Mouse FcεRI on mast cells was assayed similarly using mouse IgE. Mast cells from both transgenic and C57BL/6J mice were shown to bind to mouse IgE in flow cytometric analyses (Fig. 3, *A* and *B*). However, the amount of cell-bound mouse IgE on transgenic mast cells was less than that on the nontransgenic BMMC. Consistent with this finding, ^{125}I-IgE–binding studies also showed that mouse FcεRI receptor number on transgenic BMMC was reduced, whereas its mouse IgE–binding affinity was unchanged (Table 1). The results suggest that the cell surface expression of mouse FcεRI was reduced on BMMC from transgenic mice. This decrease in mouse FcεRI level on transgenic mast cells could be due to the competition between the human and mouse α chains in coupling with the mouse γ and/or β chains. The possibility that the human FcεRI α chain forms a chimeric receptor with mouse γ and/or β chains has also been suggested by previous transfection studies (9, 10, 26–28).

The Transgenic Human FcεRI α Chain Is Complexed with the Mouse β and γ Chains. The human FcεRI α chain has been shown in transfected cells to require at least the α and γ chains for cell surface expression (10, 26–28). In this transgenic model, proteins that are complexed with the transgenic human FcεRI α chain were analyzed in immunoprecipitation studies. To maintain the integrity of the FcεRI receptor complex, BMMC were lysed with CHAPS buffer according to the method of Kinet et al. (19). The transgenic human FcεRI or the endogenous mouse FcεRI was immunoprecipitated via its binding to human or mouse IgE, respectively. The mAb specific for the rat FcεRI β chain detected the mouse β chain in mouse FcεRI immunoprecipitates with an apparent molecular mass of 30 kD (Fig. 4). Interestingly, the mouse FcεRI β chain was also present in the transgenic human FcεRI immunoprecipitates (Fig. 4). The mouse FcεRI γ chain, which was ~10 kD in size, was detected with rabbit sera specific for the mouse FcεRI γ chain (20). Similarly, the mouse γ chain was found in both the immunoprecipitates of the transgenic human FcεRI and the mouse FcεRI (Fig. 4). The results demonstrated that the human FcεRI α chain is coupled with both the mouse β and γ chains to form a chimeric human/mouse receptor.

Normal Tyrosine Phosphorylation Triggered by Cross-linking the Transgenic FcεRI. Activation and degranulation of mast cells via FcεRI aggregation has been shown to involve early signaling events including activation of protein tyrosine kinases (29–33). The function of the transgenic FcεRI in initiating tyrosine phosphorylation in BMMC was investigated. Transgenic human FcεRI was cross-linked by first binding to anti-NIP human IgE (34), followed by interacting with the multivalent antigen NIP–BSA. Mouse endogenous FcεRI was aggregated similarly by anti-DNP mouse IgE (18) and DNP–BSA. Significant induction in tyrosine

Table 1. *Number of Human FcεRI on Transgenic BMMC and Its Binding Affinity to Human IgE*

BMMC	IgE	Receptor/cell	K_d
			nM
Transgenic	Human	4.7×10^4	6.40
	Mouse	4.1×10^4	2.93
C57BL/6J	Mouse	1.8×10^5	2.19

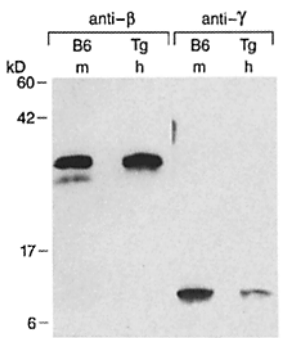

Figure 4. The transgenic human FcεRI α chain is complexed with the mouse β and γ chains. Mouse FcεRI from C57BL/6J (*B6*) BMMC or human FcεRI from transgenic (*Tg*) BMMC was immunoprecipitated with mouse IgE (*m*) or human IgE (*h*) as described in Materials and Methods. The mouse FcεRI β chain was detected with anti-rat β mAb (20), whereas the γ chain was detected by using the γ chain–specific rabbit serum (21).

phosphorylations was detected 2 min after cross-linking of mouse FcεRI on BMMC from transgenic or control C57BL/6J mice (Fig. 5). Human IgE and NIP–BSA also triggered pronounced tyrosine phosphorylations in transgenic BMMC but not in C57BL/6J BMMC, suggesting that the human IgE–induced signaling events were mediated specifically by the transgenic human FcεRI (Fig. 5). The patterns of phosphorylated proteins induced by aggregation of the transgenic human FcεRI or the endogenous mouse FcεRI appeared to be similar (Fig. 5).

Recent reports have shown that aggregation of FcεRI leads to significant tyrosine phosphorylation of the β and γ subunits, which recruit other signaling molecules for cellular activation (29, 31). Tyrosine-phosphorylated proteins in immunoprecipitates of the transgenic FcεRI were therefore examined. Proteins of ~30 and 10 kD, which correspond by size to the FcεRI β and γ chains, respectively, were barely detectable in immunoprecipitates of uncross-linked mouse FcεRI (Fig. 6). Upon aggregation of mouse FcεRI, these proteins were dramatically enhanced in tyrosine phosphorylation (Fig. 6). Cross-linking of the transgenic FcεRI also showed significant tyrosine phosphorylation of the 30 and 10 kDa proteins (Fig. 6). Human IgE does not bind to mouse FcεRI and therefore had no effect on FcεRI on nontransgenic BMMC. Taken together, the data suggest that the transgenic human FcεRI is functional in triggering mast cell activation. The coupling of mouse β and γ chains with the transgenic human α chain appears not only important for cell surface expression, but also for the proper function of the chimeric receptor.

Degranulation of Transgenic Mast Cells Triggered by Cross-linking the Human FcεRI α Chain. With this transgenic model, we address the question whether the human/mouse chimeric receptor is competent in eliciting mast cell degranulation. Mast cells were prepared from transgenic mice, and degranulation was triggered by first allowing the binding of IgE to FcεRI on mast cells, followed by cross-linking receptor-bound IgE with a multivalent antigen. Release of β-hexosaminidase upon degranulation was measured. The NIP-specific human IgE and NIP–BSA triggered a specific release of β-hexosaminidase from transgenic BMMC, but not from control C57BL/6J BMMC (Fig. 7). The DNP-specific mouse IgE and DNP–BSA induced degranulation of BMMC from both transgenic and nontransgenic mice (Fig. 7). The results indicate that the FcεRI with the transgenic human α chain is responding to human IgE, and is functional in mediating murine mast cell exocytosis. Mast cells from transgenic mice, however, can still respond to mouse IgE in exocytosis.

Passive Cutaneous Anaphylaxis of Transgenic Mice Induced by Human IgE and Antigens. To assess whether the transgenic FcεRI with the human α chain can respond to human IgE in mast cell degranulation in vivo, passive cutaneous anaphylaxis was carried out in transgenic mice. Mice were intradermally injected wtih human or mouse IgE, followed by intravenous injection of antigens and Evans blue. In transgenic mice, human IgE and its antigen NIP-BSA induced a specific leak of Evans blue into the skin at the site of intradermal injection. This blue dye leakage was not observed in control C57BL/6J mice (Fig. 8). The extravasation of the dye is a result of blood vessel dilation, which is induced by mediators released from mast cells in degranulation. Mouse monoclonal IgE and its antigen DNP–BSA caused a similar leak of Evans blue in both transgenic mice and control C57BL/6J mice (Fig. 8). The results provide a

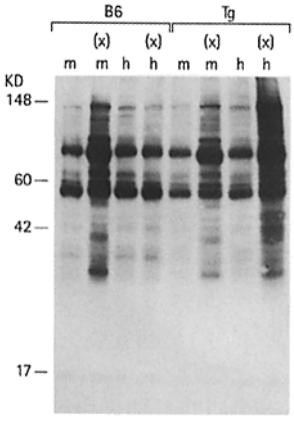

Figure 5. Tyrosine phosphorylation of cellular proteins induced by cross-linking of transgenic FcεRI. FcεRI on BMMC from C57BL/6J (*B6*) and transgenic (*Tg*) mice were cross-linked (*X*) by mouse IgE (*m*) or human IgE (*h*) plus their specific antigens as described in Materials and Methods. Uncross-linked controls were samples treated with IgE but not the antigens. Total cell lysates were prepared 2 min after cross-linking the receptors, and phosphotyrosines were detected on Western blots.

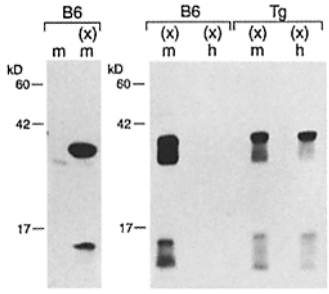

Figure 6. Tyrosine phosphorylation of the mouse β and γ chains in the transgenic FcεRI upon receptor aggregation. FcεRI on BMMC from C57BL/6J (*B6*) or transgenic (*Tg*) mice were cross-linked (*X*) by mouse IgE (*m*) or human IgE (*h*) plus their specific antigens. FcεRI was then immunoprecipitated, and phosphotyrosines were detected as described in Materials and Methods. Uncross-linked mouse FcεRI was also used as a control for comparison.

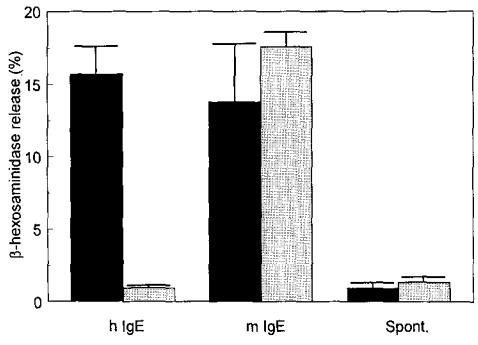

Figure 7. Human IgE plus antigens mediated degranulation of mast cells from transgenic mice. β-Hexosaminidase released from transgenic BMMC (*black column*) and nontransgenic C57BL/6J BMMC (*gray column*) were induced by human or mouse monoclonal IgE plus their specific antigens, NIP–BSA or DNP–BSA, respectively. The value of β-hexosaminidase release is the average of three experiments. Error bars indicate SEM. In nontransgenic BMMC, β-hexosaminidase release induced by human IgE is not significantly different from spontaneous release (p = 0.41 by Wilcoxin rank sum test).

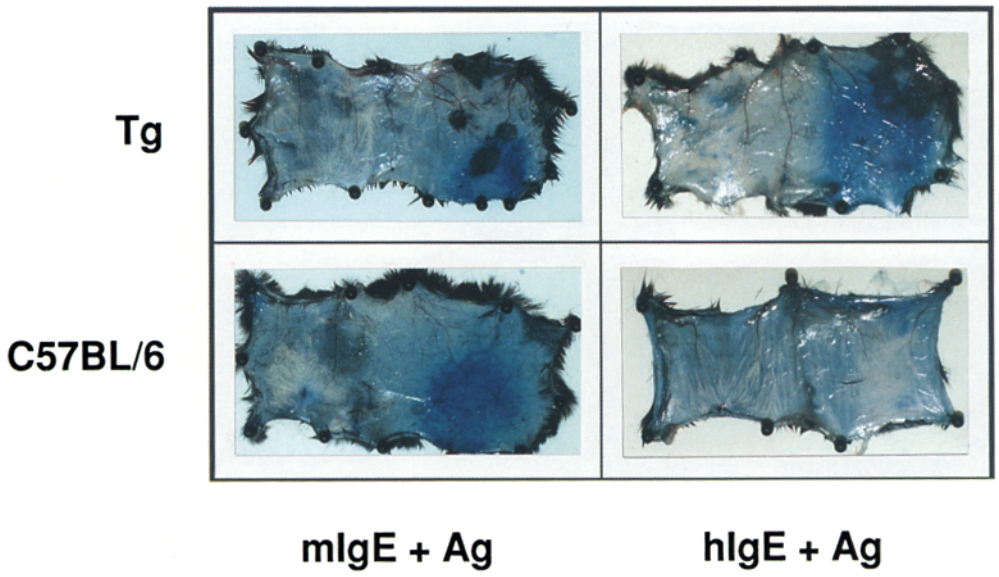

Figure 8. Human IgE plus antigen induced passive cutaneous anaphylaxis in transgenic mice. Passive cutaneous anaphylaxis in transgenic (*Tg*) and C57BL/6J control (*C57BL/6*) mice was triggered by mouse IgE (*mIgE*) or human IgE (*hIgE*) plus their specific antigens as described in Materials and Methods.

clear in vivo demonstration that transgenic mice can respond to human IgE in anaphylaxis.

Discussion

Human FcεRI expression on the cell surface is known to require at least the α and γ chains (10, 26–28). The ability of the γ chain to allow surface expression of the FcεRI α chain is analogous to the role of the ζ chains in the expression of the TCR–CD3 complex, although the molecular mechanism of the FcεRI assembly is still unclear. Both the FcεRI α and γ chains are highly conserved at the transmembrane portions, which are suggested to be important for proper assembly of the FcεRI receptor complex (10, 28). In contrast, the role of the β chain for cell surface expression and activation of human FcεRI has been unclear. Questions have therefore been raised regarding to the importance of the FcεRI β chain in humans and the possible difference in FcεRI receptor complex between humans and rodents. We have demonstrated here in a transgenic model that the transgenic FcεRI, which elicits normal mast cell activation and degranulation, is composed of the human α chain and the mouse β and γ chains. The data suggest that the β chain may play a role in the biological functions of FcεRI. The importance of the β chain has also been suggested by the finding that it is also associated with the IgG Fc receptor type III, which mediates effector functions similar to that of FcεRI (35). A correlation of mutations on the FcεRI β chain gene with atopic dermatitis has also been reported recently (36, 37).

The transgenic FcεRI in which only the α chain was of human origin was shown to bind to human IgE specifically. This is consistent with previous in vitro studies showing that the α chain confers entirely the IgE-binding specificity, whereas the β and γ chains are responsible for signaling events (6, 11, 29, 31, 38, 39). The extracellular portion of the FcεRI α chain has been shown to display less conservation between species (10). This structural diversity could be responsible for the species specificity seen in IgE–FcεRI interaction.

The proper assembly and coordination of the different subunits of FcεRI is essential for its function in triggering mast cell degranulation (1). In this regard, the FcεRI α chain receives external signals through IgE binding, whereas the FcεRI γ chain plays a critical role in signal transduction (11, 29, 31, 38, 39). Although the human FcεRI α chain in association with mouse γ chains has been shown to trigger limited early signaling events in mast cell lines, the induction of cellular degranulation has not been demonstrated (26, 39, 40). We showed with this transgenic model that the human/mouse chimeric FcεRI is functional in mediating mast cell activation and degranulation. The results suggest that the proper conformation of the receptor complex is maintained in the transgenic FcεRI.

Allergic diseases affect 20% of the population and are an important cause of morbidity and mortality. The cell type–specific expression and the key role in mast cell exocytosis make FcεRI an attractive drug target for treatment of IgE-dependent allergies. However, animal studies are limited by the species specificity in IgE–FcεRI interaction. We have demonstrated here in a transgenic model that the human FcεRI α chain not only confers the specificity in human IgE binding but also can reconstitute a functional FcεRI to trigger mast cell degranulation in vitro and in vivo. These transgenic mice with a "humanized" FcεRI could be a valuable model for developing drugs that block human IgE from binding to its receptor.

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Address correspondence to Dr. Wai-Ping Fung-Leung, The. R.W. Johnson Pharmaceutical Research Institute, 3535 General Atomics Court, Suite 100, San Diego, CA 92121.

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References

1. Metzger, H. 1992. The receptor with high affinity for IgE. *Immunol. Rev.* 125:37–48.
2. Metzger, H., G. Alcaraz, R. Hohman, J.P. Kinet, V. Pribluda, and R. Quarto. 1986. The receptor with high affinity for immunoglobulin E. *Annu. Rev. Immunol.* 4:419–470.
3. Serafin, W.E., and Austen, K.F. 1987. Mediators of immediate hypersensitivity reactions. *N. Engl. J. Med.* 317:30–34.
4. Stevens, R.L., and K.F. Austen. 1989. Recent advances in the cellular and molecular biology of mast cells. *Immunol. Today.* 10:381–386.
5. 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.* 75:969–976.
6. Hakimi, J., C. Seals, J.A. Kondas, L. Pettine, W. Danho, and J. Kochan. 1990. The α subunit of the human IgE receptor (FcεR1) is sufficient for high affinity IgE binding. *J. Biol. Chem.* 265:22079–22081.
7. Riske, F., J. Hakimi, M. Mallamaci, M. Griffin, B. Pilson, N. Tobkes, P. Lin, W. Danho, J. Kochan, and R. Chizzonite. 1991. High affinity human IgE receptor (FcεR1). *J. Biol. Chem.* 266:11245–11251.
8. Blank, U., C. Ra, and J.P. Kinet. 1991. Characterization of truncated α chain products from human, rat and mouse high affinity receptor for immunoglobulin E. *J. Biol. Chem.* 266: 2639–2646.
9. Blank, U., C. Ra, L. Miller, and J.P. White. 1989. Complete structure and expression in transfected cells of high affinity IgE receptor. *Nature (Lond.).* 337:187–189.
10. Ra, C., M.H. Jouvin, and J.P. Kinet. 1989. Complete structure of the mouse mast cell receptor for IgE (FcεR1) and surface expression of chimeric receptors (rat-mouse-human) on transfected cells. *J. Biol. Chem.* 264:15323–15327.
11. Ravetch, J.V., and J.P. Kinet. 1991. Fc receptors. *Annu. Rev. Immunol.* 9:457–492.
12. Gounni, A.S., B. Lamhiared, K. Ochiai, Y. Tanaka, E. Delaporte, A. Capron, J.P. Kinet, and M. Capron. 1994. High affinity IgE receptor on eosinophils is involved in defence against parasites. *Nature (Lond.).* 367:183–186.
13. Maurer, D., A. Rieger, O. Kilgus, B. Reininger, K. Ochiai, J.P. Kinet, and G. Stingl. 1994. Expression of functional high affinity immunoglobulin E receptors (FcεRI) on monocytes of atopic individuals. *J. Exp. Med.* 179:745–750.
14. Wang, B., A. Rieger, O. Kilgus, K. Ochiai, D. Maurer, D. Fodinger, J.P. Kinet, and G. Stingl. 1992. Epidermal Langerhans cells from normal human skin bind monomeric IgE via FcεR1. *J. Exp. Med.* 175:1353–1365.
15. 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 (FcεR1). *J. Exp. Med.* 175: 1285–1290.
16. Pang, J., G.R. Taylor, D.G. Munroe, A. Ishaque, W.-P. Fung-Leung, C.Y. Lau, F.-T. Liu, and L. Zhou. 1993. Characterization of the gene for the human high affinity IgE receptor (FcεR1) α-chain. *J. Immunol.* 151:6166–6174.
17. Razin, E., C. Cordon-Cardo, and R.A. Good. 1981. Growth of a pure population of mouse mast cells in vitro with conditioned medium derived from concanavalin A-stimulated splenocytes. *Proc. Natl. Acad. Sci. USA.* 78:2559–2561.
18. Liu, F.-T., J.W. Bohn, E.L. Ferry, H. Yamamoto, C.A. Molinaro, L.A. Sherman, N.R. Klinman, and D.H. Katz. 1980. Monoclonal dinitrophenyl-specific murine IgE antibody: preparation, isolation and characterization. *J. Immunol.* 124:2728–2737.
19. Kinet, J.-P., G. Alcaraz, A. Leonard, S. Wank, and H. Metzger. 1985. Dissociation of the receptor for immunoglobulin E in mild detergents. *Biochemistry.* 24:4117–4124.
20. Kurosaki, T., and J.V. Ravetch. 1989. A single amino acid in the glycosylphosphatidylinositol attachment domain determines the membrane topology of FcγRIII. *Nature (Lond.).* 342:805–807.
21. Yen, A., F.-T. Liu, K.E. Barrett, and I. Gigli. 1993. Alterations in FcεRI induced by photoporthyrin plus long wavelength ultraviolet light in mouse bone marrow derived mast cells. *J. Immunol.* 151:1003–1011.
22. Schwartz, L.B., K.F. Austen, and S.I. Wasserman. 1979. Immunologic release of β-hexosaminidase and β-glucuronidase from purified rat serosal mast cells. *J. Immunol.* 123:1445–1450.
23. Saloga, J., H. Renz, G. Lack, K.L. Bradley, J.I. Greenstain, G. Larsen, and E.W. Gelfand. 1993. Development and transfer of immediate cutaneous hypersensitivity in mice exposed to aerosolized antigen. *J. Clin. Invest.* 91:133–140.
24. Capron, A., J.P. Dessaint, M. Capron, M. Joseph, J.C. Ameisen, and A.B. Tonnel. 1986. From parasites to allergy: a second receptor for IgE. *Immunol. Today.* 7:15–18.
25. Ravetch, J., and J.-P. Kinet. 1991. Fc receptors. *Annu. Rev. Immunol.* 9:457–492.
26. Miller, L., G. Alber, N. Varin-Blank, R. Ludowyke, and H. Metzger. 1990. Transmembrane signalling in p815 mastocytoma cells by transfected IgE receptors. *J. Biol. Chem.* 265: 12444–12553.
27. Kuster, H., H. Thompson, and J.P. Kinet. 1990. Characterization and expression of the gene for the human Fc receptor gamma subunit. Definition of a new gene family. *J. Biol. Chem.* 265:6448–6452.
28. Varin-Blank, N., and H. Metzger. 1990. Surface expression of mutated subunits of the high affinity mast cell receptor for IgE. *J. Biol. Chem.* 265:15685–15694.
29. Eiseman, E., and J.B. Bolen. 1992. Engagement of the high-affinity IgE receptor activates src protein-related tyrosine kinases. *Nature (Lond.).* 355:78–80.
30. Minoguchi, K., M. Benhamou, W.D. Swain, Y. Kawakami, T. Kawakami, and R.P. Siraganian. 1994. Activation of protein tyrosine kinase $p75^{syk}$ by FcεRI aggregation in rat baso-

philic leukemia cells. *J. Biol. Chem.* 269:16902–16908.
31. Jouvin, M.-H.E., M. Adamczewski, R. Numerof, O. Letourneur, A. Valle, and J.-P. Kinet. 1994. Differential control of the tyrosine kinases Lyn and Syk by the two signaling chains of the high affinity immunoglobulin E receptor. *J. Biol. Chem.* 269:5918–5925.
32. Yamashita, T., S.-Y. Mao, and H. Metzger. 1994. Aggregation of the high-affinity IgE receptor and enhanced activity of $p53/56^{lyn}$ protein-tyrosine kinase. *Proc. Natl. Acad. Sci. USA.* 91:11251–11255.
33. Fukamachi, H., N. Yamada, T. Miura, T. Kato, M. Ishikawa, E. Gulbins, A. Altman, Y. Kawakami, and T. Kawakami. 1994. Identification of a protein, SPY75, with repetitive helix-turn-helix motifs and an SH3 domain as a major substrate for protein tyrosine kinases activated by FcεRI cross-linking. *J. Immunol.* 152:642–652.
34. Neuberger, M.S., G.T. Williams, E.B. Mitchell, S.S. Jouhal, J.G. Flanagan, and T.H. Rabbitts. 1985. A hapten-specific chimeric IgE antibody with human physiological effector function. *Nature (Lond.).* 314:268–270.
35. Kurosaki, T., I. Gander, U. Wirthmueller, and J.V. Ravetch. 1992. The β subunit of the FcεRI is associated with the FcγRIII on mast cells. *J. Exp. Med.* 175:447–451.
36. Ravetch, J.V. 1994. Atopy and Fc receptors: mutation is the message? *Nature Genetics.* 7:117–118.
37. Shirakawa, T., A. Li, M. Dubowitz, J.W. Dekker, A.E. Shaw, J.A. Faux, C. Ra, W.O.C.M. Cookson, and J.M. Hopkin. 1994. Association between atopy and variants of the β subunits of the high-affinity immunoglobulin E receptor. *Nature Genetics.* 7:125–129.
38. Kinet, J.P. 1992. The gamma-zeta dimers of Fc receptors as connectors to signal transduction. *Curr. Opin. Immunol.* 4:43–48.
39. Alber, G., L. Miller, C.L. Jelsema, N. Varin-Blank, and H. Metzger. 1991. Structure-function relationships in the mast cell high affinity receptor for IgE. *J. Biol. Chem.* 266:22613–22620.
40. Taudou, G., N. Varin-Blank, H. Jouin, F. Marchand, A. Weyer, and U. Blank. 1993. Expression of the alpha chain of human FcεRI in transfected rat basophilic leukemia cells: functional activation after sensitization with human mite-specific IgE. *Int. Arch. Allergy Immunol.* 100:344–350.
41. Kishi, K. 1985. A new leukemia cell line with Philadelphia chromosome characterized as basophil precursors. *Leuk. Res.* 9:381–390.