

Rapid desensitization of humanized mice with anti-human Fc ϵ RI α monoclonal antibodies



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Background: Anaphylaxis is classically mediated by allergen cross-linking of IgE bound to the α chain of Fc ϵ RI, the mast cell/basophil high affinity IgE receptor. Allergen cross-linking of the IgE/Fc ϵ RI complex activates these cells, inducing release of disease-causing mediators, cytokines, and enzymes. We previously demonstrated that IgE-mediated anaphylaxis could be safely prevented in wild-type BALB/c mice by rapid desensitization with anti-mouse Fc ϵ RI α mAb.

Objective: This study sought to use humanized mice to extend these results to humans.

Methods: We actively immunized huFc ϵ RI α /F709 mice, which express human (hu) instead of mouse Fc ϵ RI α and a mutant IL-4 receptor that lacks inhibitory function. We passively immunized huFc ϵ RI α mice, as well as human cord blood-reconstituted reNSGS mice, which are immune-deficient, produce mast cell-stimulating human cytokines, and develop numerous human mast cells. For desensitization, we used anti-huFc ϵ RI α mAbs that bind Fc ϵ RI α regardless of its association with IgE (noncompeting mAbs), and/or mAbs that compete with IgE for huFc ϵ RI α binding (competing mAbs). Anaphylaxis was induced by intravenous injection of antigen or anti-huIgE mAb.

Results: Anti-huFc ϵ RI α mAb rapid desensitization was safer and more effective than allergen rapid desensitization and suppressed anaphylaxis more rapidly than omalizumab or

ligelizumab. Rapid desensitization of naïve, IgE-sensitized huFc ϵ RI α mice and huFc ϵ RI α /F709 mice that were egg-allergic with anti-Fc ϵ RI α mAbs safely removed >98% of IgE from peritoneal mast cells and completely suppressed IgE-mediated anaphylaxis. Rapid desensitization of reNSGS mice with anti-Fc ϵ RI α mAbs also safely removed ~98% of mast cell IgE and prevented IgE-mediated anaphylaxis.

Conclusions: Rapid desensitization with anti-Fc ϵ RI α mAbs may be a safe, effective, and practical way to prevent IgE-mediated anaphylaxis. (*J Allergy Clin Immunol* 2020;145:907-21.)

Key words: Anaphylaxis, antibody, IgE, mouse

Anaphylaxis is “a serious systemic allergic reaction that is rapid in onset and may cause death.”¹ Its most serious, potentially lethal manifestations are cardiovascular collapse and severe bronchospasm; its most common elicitors are foods, drugs, and venoms.²⁻⁴ The prevalence of anaphylaxis in the United States “is at least 1.6% and probably higher” and close to 34% of Americans who had an episode of this disorder visited a hospital.⁵ The only treatment for anaphylaxis is symptomatic, and there is no approved prophylaxis other than allergen avoidance.

Anaphylaxis can be induced by >1 pathogenic mechanism. Antibody-dependent mechanisms include the classical pathway, in which anaphylaxis is induced by antigen (Ag) cross-linking of Ag-specific IgE that has bound to Fc ϵ RI, the high affinity IgE receptor on mast cells and basophils,⁶⁻⁸ and an alternative pathway, in which anaphylaxis is induced by antigen/IgG immune complex cross-linking of Fc ϵ Rs on basophils, neutrophils, macrophages, and possibly mast cells.⁹ In the classical pathway, crosslinking of Fc ϵ RI initiates mast cell degranulation, with release of preformed vasoactive mediators, enzymes, and cytokines, as well as the synthesis and secretion of additional mediators and cytokines. Together, these mediators, cytokines, and enzymes cause most of the features of anaphylaxis through their effects on epithelial cells, vascular endothelial cells, smooth muscle cells, mast cells, basophils, eosinophils, and additional cell types.¹⁰

Although exposing mast cells and basophils that have been sensitized to Ag-specific IgE to a sufficient concentration of the cognate antigen can induce severe disease, including anaphylaxis, exposure to smaller quantities of antigen can decrease the responsiveness of these cells to subsequent exposure to larger quantities of the same antigen.¹¹⁻¹⁴ This phenomenon is the basis for a process known as rapid desensitization or rush desensitization, which is most commonly used to treat individuals who require a drug to which they are allergic.^{14,15} In this process,

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Disclosure of potential conflict of interest: M. V. Khodoun, S. C. Morris, C. Potter, A. B. Herr, and F. D. Finkelman have rights in a patent for rapid desensitization with anti-Fc ϵ RI α mAbs that was awarded to the University of Cincinnati and could benefit from the licensing of this patent. F. D. Finkelman is a principal in a start-up company that is trying to commercialize rapid desensitization with anti-Fc ϵ RI α monoclonal antibodies. The rest of the authors declare that they have no relevant conflicts of interest.

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Abbreviations used

γ_c :	Cytokine receptor common γ chain, also known as IL-2 receptor γ chain
Ag:	Antigen
EW:	Egg white
EYP:	Egg yolk plasma
HNA:	HBSS plus 10% newborn bovine serum plus 0.2% NaN_3
hu:	Human
NOD:	Nonobese diabetic
NSGS mice:	NOD background SCID mice that lack γ_c and express transgenic human GM-CSF, IL-3, and stem cell factor (Kit ligand)
OVA:	Ovalbumin
reNRGS mice:	Mice that are T cell–depleted human cord blood–reconstituted with NOD background
reNSGS mice:	NSGS mice that have been reconstituted with T cell–depleted human cord blood cells
OG:	Oral gavage
SCID:	Severe combined immunodeficiency
SCF:	Stem cell factor
SPR:	surface plasmon resonance
THSDT:	Tukey honest significant difference test
TNP:	Trinitrophenyl
WT:	Wild-type

patients with allergy are sequentially exposed, over a period of hours, to increasing doses of the disease-causing drug, starting with a dose that is too small to cause disease and finishing with a therapeutic dose of the same drug. Theoretically, at least, the same process could be used to desensitize individuals to any allergen.

Because atopic individuals are frequently allergic to multiple allergens,^{16,17} it would be advantageous to have a procedure that desensitized their mast cells and basophils to all allergens, rather than a single allergen or allergen-containing substance. With this in mind, we evaluated the possibility of replacing an allergen with an IgG mAb to Fc ϵ RI α for rapid desensitization of mice that had been passively or actively sensitized with Ag-specific IgE.¹¹ The results of this study demonstrated that (1) mice could be rapidly desensitized with MAR-1, a hamster IgG mAb that selectively binds mouse Fc ϵ RI α that is not associated with IgE; (2) although a single large dose of MAR-1 caused anaphylaxis, administering MAR-1 by a rapid desensitization approach did not cause hypothermia, decreased movement, or any other sign of disease that we were able to observe; (3) rapid desensitization with MAR-1 induced short-lived mast cell anergy; (4) continued treatment with MAR-1 for several days removed all IgE from mast cells; (5) once accomplished, loss of IgE could be safely maintained by repeated, large doses of MAR-1; (6) when complete, rapid desensitization with MAR-1 completely suppressed IgE-mediated anaphylaxis in passive and active models; (7) rapid desensitization with MAR-1 was safer and its effects longer lasting than rapid desensitization with an antigen in mice that had been actively sensitized to that antigen; and (8) rapid desensitization with MAR-1 was Ag-nonspecific.

These observations encouraged us to initiate studies to determine whether a similar approach could rapidly desensitize humans. To evaluate this, we have investigated the ability of mouse mAbs to human (hu) Fc ϵ RI α to desensitize 3 types of mice: (1) huFc ϵ RI α mice, in which the mouse Fc ϵ RI α gene has been deleted and a huFc ϵ RI α transgene has been expressed, so

that they express functional, chimeric Fc ϵ RI (huFc ϵ RI α chain/mouse Fc ϵ RI β and γ chains) on their mast cells and basophils;¹⁸ (2) huFc ϵ RI α /F709 mice, which additionally express a mutant form of the IL-4R α that is incapable of negative signaling; this T_H2 biases these mice, making them particularly susceptible to allergic reactions;¹⁹ and (3) reNSGS, which are NSGS mice that have been reconstituted with T cell–depleted human cord blood cells, and reNRGS mice, which are T cell–depleted human cord blood–reconstituted with nonobese diabetic (NOD) background, severe combined immunodeficiency (SCID) or RAG1-deficient mice that lack the cytokine receptor common γ chain (γ_c) and express transgenes that encode huIL-3, huGM-CSF, and human stem cell factor (huSCF, the Kit ligand).²⁰ In our studies with these mice, we have used 2 types of anti-huFc ϵ RI α mAbs, 2 mAbs that resemble MAR-1 by only binding to Fc ϵ RI that is not associated with IgE (competing mAbs), and 2 mAbs that bind to huFc ϵ RI α regardless of its association with IgE (noncompeting mAbs). This was done in the hope that noncompeting mAbs could remove IgE and Fc ϵ RI α from mast cells and basophils more rapidly than was accomplished by MAR-1 in conventional mice. The results of our studies establish a rapid desensitization protocol that safely removes nearly all IgE from humanized and human mast cells and basophils and prevents IgE-mediated anaphylaxis.

METHODS

Mice

BALB/c mice were purchased from Taconic (Hudson, NY) or bred in the Cincinnati Children's Hospital Medical Center (Children's) animal facility. Fc ϵ RI α -deficient mice on a BALB/c background¹⁸ were a gift of Jean-Pierre Kinet (Harvard University, Cambridge, Mass) and bred in our facility. IL-4 receptor F709ITIM mutant mice on a BALB/c background¹⁹ (C.129X1-IL4ra^{tm3.1Tch}/J) were a gift of Talal Chatila and Hans Oettgen (Harvard). F709Tg/huFc ϵ RI α Tg/muFc ϵ RI α -deficient mice on a BALB/c genetic background were generated by in-house crossbreeding with PCR typing. Human IL-3/SCF/GM-CSF transgenic mice on an immune-deficient NOD/LtSz-SCID IL-2RG^{-/-} (NSGS) or NOD/Rag1^{-/-}/IL-2RG^{-/-} (NRGS) background were obtained from a core at Children's and engrafted with T cell–depleted human cord blood cells as previously described.^{20,21} Mice of both sexes who were 8 to 12 weeks old were used. Cages of mice were randomly assigned to groups (without a specific randomization protocol), with the exception that groups were age- and sex-matched. Researchers were not blinded as to group. Animal work was approved by the Cincinnati Children's Hospital Research Foundation Institutional Animal Care and Use Committee.

Reagents

Ovalbumin (OVA) and BSA were purchased from Sigma (St Louis, Mo). Trinitrophenyl (TNP)-OVA and TNP-BSA were prepared by haptenating OVA or BSA, respectively, with 2,4,6-TNP- ϵ -aminocaproyl-O-succinamide (Biosearch Technologies, Novato, Calif). To prepare egg antigen, egg white (EW) and egg yolk, removed under sterile conditions from 12 fresh chicken eggs, were pooled separately. The EW was dialyzed against distilled water and centrifuged for 15 minutes at 3900g to remove precipitate, then analyzed for protein concentration using a Pierce BCA Protein Assay Kit (Thermo Fisher Scientific, Waltham, Mass), then pressure concentrated with a stirred ultrafiltration cell (Millipore, Temecula, Calif) with a 30-kDa cutoff Diaflo membrane (Sigma-Aldrich, St Louis, Mo). The yolks were diluted 1:1 with 0.15 mol NaCl and centrifuged to remove egg yolk granules. The supernatant (egg yolk plasma [EYP]) was added to the EW while the EW was being concentrated to create a mixture that was composed of the EYP from 3 eggs and EW from 12 eggs and had a final concentration of 333 mg of EW protein/mL.

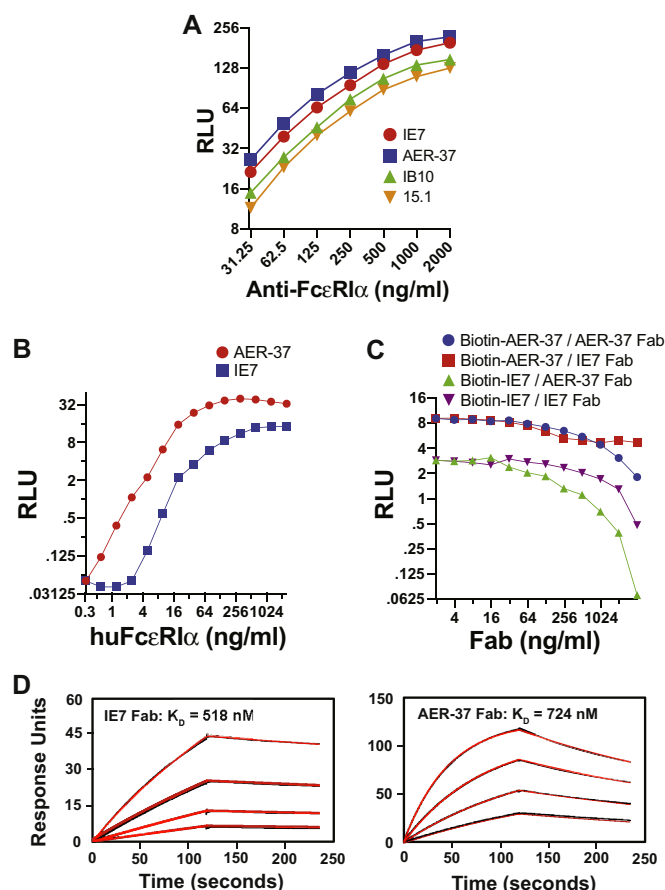


FIG 1. Evaluation of anti-FcεR1α mAb affinity and avidity. **A**, Microtiter plate wells were coated with huFcεR1α external domain (5 μg/mL). After blocking, serial dilutions of intact mouse IE7, AER-37, IB10, and 15.1 were added to wells and detected with biotin-labeled anti-mouse κ, followed by enzyme-streptavidin. **B**, Microtiter plate wells were coated with huFcεR1α external domain at the concentrations shown plus BSA (total protein concentration was always 5 μg/mL). After blocking, intact biotin-human IgG₁ AER-37 or intact biotin-mouse IE7 at 8 ng/mL was added to wells, followed by horseradish peroxidase-streptavidin. **C**, Microtiter plate wells were coated with huFcεR1α external domain (20 ng/mL) plus BSA (4.98 μg/mL). After blocking, 8 ng/mL of intact biotin-human IgG₁ AER-37 or intact biotin-mouse IE7, supplemented with Fab fragments of IE7 or human IgG₁ AER-37 at the concentrations shown, were added to the wells, followed by horseradish peroxidase-streptavidin and substrate. **D**, SPR kinetic data; raw data are shown in black with the fitted curves superimposed in red. Left, IE7 Fab binding to FcεR1α ectodomain. IE7 Fab was injected at concentrations of 3, 1.5, 0.75, and 0.375 μmol and analyzed using a 1:1 model of binding. Right, AER-37 Fab binding to FcεR1α ectodomain. AER-37 Fab was injected at concentrations of 5, 2.5, 1.25, and 0.625 μmol and analyzed using a 1:1 model of binding with a mass transport term. RLU, Relative luminescence units.

Antibodies

The hybridoma that produces mAb 15.1 anti-huFcεR1α mAb was a gift of Jean-Pierre Kinet (Harvard).²² This hybridoma was grown as ascites in pristane-primed athymic nude mice; the mAb was purified from ascites by ammonium sulfate precipitation followed by DE-52 cellulose ion exchange chromatography. For some experiments, it was further purified by adsorption to and elution from staphylococcal protein A- or protein G-agarose. The anti-huFcεR1α mAb AER-37 (also called CRA-1)²³ was purchased from BioLegend (San Diego, Calif). To generate additional anti-huFcεR1α mAbs, FcεR1α-deficient mice (BALB/c background) were immunized 3 times intraperitoneally at 2-week intervals with 20 μg of the 176 N-terminal amino acid human FcεR1α ectodomain (produced in-house and validated

by SDS-PAGE and Western blotting) in 50% alum adjuvant. Once high titers of mouse IgG₁ anti-human FcεR1α were detected by ELISA, the mice were injected intravenously with 2 μg of human FcεR1α ectodomain; 2 days later, their splenocytes were fused with a nonsecreting mouse plasmacytoma cell and cloned. Five mouse IgG₁ anti-human FcεR1α ectodomain mAb-secreting clones were selected by ELISA, followed by flow cytometric evaluation of their ability to stain peritoneal mast cells from mice that expressed human but not mouse FcεR1α and their failure to stain peritoneal mast cells from normal BALB/c mice. Selected clones, including the clones IE7 and IB10 that are used in this paper, were expanded for mAb generation and grown as ascites in pristane-primed BALB/c mice. IE7 and IB10 mAbs were purified from ascites by ammonium sulfate fractionation followed by DEAE-Sephadex (Sigma-Aldrich) chromatography.

IB10 and 15.1 are competing mAbs, that is, they directly compete with IgE for binding to FcεR1α. This indicates that they likely bind to the second Ig-like domain of FcεR1α, because this domain is responsible for IgE binding.²⁴ AER-37 has previously been shown not to block IgE binding to FcεR1α (ie, it is a noncompeting mAb) and to bind to FcεR1α's first Ig-like domain;²⁵ IE7 probably binds to the same domain because it and AER-37 cross-block each other's binding to FcεR1α (data not shown).

Preparation of mAb Fab fragments

Fab fragments of the anti-huFcεR1α mAbs IE7, a huIgG₁ variant of AER-37, IB10, and 15.1 were generated with a Pierce Mouse IgG₁ Fab preparation kit (Thermo Fisher Scientific) according to the manufacturer's instructions. The incubation time of protein G-agarose affinity purified mAbs with particle-bound ficin was 4 hours for IE7, IB10, and 15.1 (all mouse IgG₁ mAbs) and 2 hours for AER-37 (a mouse IgG₂b mAb). Fab fragment purify, including the absence of intact mAb and F(ab')₂ fragments, was checked by reduced and unreduced SDS-PAGE.

Detection of systemic anaphylaxis

Anaphylaxis was detected primarily as a change in mouse activity and the development of hypothermia.⁶ Changes in activity were noted. Temperature was measured every 5 to 15 minutes with a rectal probe. Previous publications have shown that the development of hypothermia correlates with mast cell degranulation in murine IgE-mediated anaphylaxis by demonstrating (1) a correlation between degree of hypothermia and mouse mast cell protease 1 (MMCP-1) responses;²⁶⁻²⁸ (2) the failure of mast cell-deficient mice to develop hypothermia in response to cross-linking of IgE;⁶ and (3) the importance of histamine in IgE-mediated anaphylaxis.⁶

Measurement of MMCP-1 levels

Serum levels of MMCP-1 were measured in blood drawn 3 or 4 hours after challenge with an ELISA kit (eBioscience, San Diego, Calif), according to the manufacturer's protocol.

Active sensitization of huFcεR1α/F709 mice

After shaving their dorsal skin with electric clippers, mice were sensitized epicutaneously by applying a gauze pad soaked with egg antigen that contained 100 μg of EW under an occlusive dressing. This pad was changed weekly for 3 weeks. Mice were then inoculated by oral gavage (OG) twice a week with egg antigen that contained 100 mg of EW until they reproducibly developed a temperature drop of at least 1°C and/or diarrhea during the hour after OG inoculation.

Rapid desensitization

Unless otherwise indicated, rapid desensitization was usually accomplished by hourly intraperitoneal injections of doubling doses of ≥1 anti-FcεR1α mAb, starting at 0.025 μg and ending with 100 μg. In mice that were particularly sensitive to the development of anaphylaxis, a gentler, 3-day desensitization protocol was used.

TABLE I. SPR evaluation of IE7 and AER-37 affinity

mAb	K_{on} (ms^{-1})	K_{off} (s^{-1})	K_D (nmol)	R_{max} (RU)	Residual SD
mIE7 Fab	1.332×10^3	6.90×10^{-4}	518 ± 4	121.5	0.354
AER-37 Fab	4.127×10^3	2.99×10^{-3}	724 ± 1	142.0	0.461

RU, Response units.

Generation of humanized IgE anti-TNP mAb

RNA was extracted from cells of the hybridoma 1B7.11, which secretes mouse IgG₁ anti-TNP mAb, using the Agilent Absolutely RNA Miniprep kit (Santa Clara, Calif) according to the manufacturer's directions. cDNA was synthesized from this RNA with the Promega ImProm-II Reverse transcription system (Madison, Wis) using both random primer and oligo (dT) primer. The V_H and V_K regions were PCR-amplified from the cDNA using DreamTaq (Thermo Fisher Scientific). Primers Bi4 (HindIII C_{yl} 5'-CCAGGGGC CAGTGGATAGACAAGCTTGGGTGTCGTTT) and Bi3c (PstI, PvuII V_H domain, AGGTSMAGCTGCAGSAGTCWGG) were used for the heavy chain, and primers Bi5 (BamHI C_k domain, GGGAAGATGGATC CAGTTGGTGCAGCATCAGC) and Bi7 (EcoRV V_K domain, GGTGA TATCWTGMTGACCAAWCTCCACTCTC) were used for the light chain. The PCR products were cloned into pCR2.1-TOPO using the TOPO-TA Cloning kit (Invitrogen, Thermo Fisher Scientific) according to the manufacturer's directions. Products in pCR2.1-TOPO were sequenced using M13R primer. The anti-TNP V_H region was amplified with PCR and primers that added EcoRV and NheI restriction enzyme sites to the 5' and 3' ends, respectively. This product was digested and ligated into pFUSEss-CHiG-hE (InvivoGen, San Diego, Calif) using the same enzymes. PCR was used to amplify a heavy chain construct consisting of HindIII-GCCGCCACC (Kozak)—IL-2 signal sequence—anti-TNP V_H—human C_ε—Stop—Stop—BstBI from this pFUSE plasmid (InvivoGen). The construct was digested with HindIII and BstBI and ligated into the pEE6.4 vector (Lonza Group, Basel, Switzerland). The NEBuilder HiFi DNA Assembly kit (New England Biolabs, Ipswich, Mass) was used to assemble the anti-TNP V_K region into a pEE12.4 vector (Lonza) already containing the IL-2 signal sequence and human C_k. The 2 plasmids encoding the separate κ and ε chains were digested with NotI and BamHI and ligated to make a single pEE12.4 vector containing both chains as well as the glutamine synthetase (GS) mammalian selection marker, with Lonza's GS Gene Expression System. The resulting plasmid was digested with PvuI and transfected into NS0 cells (Lonza). Transfected cells were seeded at low density in 96-well plates to obtain clones derived from single colonies. Clones were identified visually and then selected based on the amount of IgE detected in the culture medium by ELISA. NS0 cells stably expressing anti-TNP IgE were injected intraperitoneally into Pristane-primed BALB/c mice. Twenty days later, ascites fluid was harvested. The fluid was clarified and sterilized by sequential filtration with 0.8-, 0.45-, and 0.22-μm filters. IgE was quantified using the human IgE ELISA Ready-SET-Go! Kit (eBioscience).

Human IgG₁ AER-37 DNA cloning

The variable regions of AER-37 light and heavy chain genes were synthesized for us by GeneArt (Thermo Fisher Scientific) and cloned into pFUSE2ss-CLiG-hk and pFUSEss-CHiG-hGI (InvivoGen), respectively, using restriction digestion. Ig light and heavy chain genes (including signal sequence) were then cloned into pEE12.4 and pEE6.4 (Lonza) also using restriction digestion. pEE12.4 and pEE6.4 vectors were digested with BamHI and NotI and ligated together to create a plasmid that contained both immunoglobulin light and heavy chains and the glutamine synthetase selection marker.

Ligelizumab DNA cloning

The amino acid sequences for the ligelizumab heavy and light chains were obtained from World Health Organization's *Drug Information*, volume 26, and reverse translated into DNA sequences. The DNA sequences were codon-optimized for *Cricetulus griseus*

expression and synthesized for us as double-stranded fragments by GeneArt. The ligelizumab heavy and light chain genes were cloned into pEE6.4 and pEE12.4, respectively, using NEBuilder HiFi DNA Assembly Master Mix. These 2 plasmids were then PCR-amplified and assembled together using the same reagent, generating a plasmid that contained ligelizumab immunoglobulin light and heavy chains and the glutamine synthetase selection marker.

Stable cell line generation

pEE12.4 vectors containing immunoglobulin light and heavy chains were digested with PvuI and transfected into CHO-K1 cells using Lipofectamine LTX (Thermo Fisher Scientific) according to the manufacturer's directions. Stably transfected cells were selected and propagated in medium lacking glutamine and containing 25 μmol methionine sulfoximine to select for cells expressing high levels of glutamine synthetase. Transfected cells were seeded at low density in 96-well plates to obtain clones derived from single colonies. Clones were identified visually and then selected for the presence of a relatively large amount of human IgG₁ in the culture medium, as detected by ELISA.

Recombinant protein production

CHO-K1 cells stably expressing recombinant AER-37 huIgG₁ or ligelizumab were grown in glutamine-free medium containing 25 μmol methionine sulfoximine and 5% dialyzed ultra-low IgG fetal bovine serum in roller bottles for 49 days. Cell culture medium was collected every 7 days and replaced with fresh medium. Conditioned medium was concentrated and dialyzed into 50 mmol Tris, 150 mmol NaCl, pH 7.0. This material was adsorbed to GammaBind-Plus Sepharose (GE Healthcare Life Sciences, Marlborough, Mass); IgG was eluted with Gentle Ag/Ab Elution Buffer (Thermo Fisher Scientific), dialyzed into 150 mmol NaCl, and concentrated using an Amicon (Sigma-Aldrich) stirred cell.

Mast cell preparation

To obtain a cell population enriched in peritoneal mast cells, peritoneal lavage was collected by paracentesis after intraperitoneal injection of 10 mL of sterile HBSS supplemented with 5% newborn bovine serum. Cells were plated in 25-cm² cell culture flasks (Corning, Corning, NY) for 2 hours at 37°C with 5% CO₂. Nonadherent cells were collected and washed with HBSS plus 10% newborn bovine serum. To prepare a cell population that contained basophils, peripheral blood obtained by tail vein incision was collected in microtainers with K₂EDTA (BD Biosciences, San Jose, Calif). Red blood cells were lysed with ammonium-chloride-potassium lysis buffer; cells were washed twice with HBSS and resuspended in HBSS plus 10% newborn bovine serum plus 0.2% NaN₃ (HNA). Spleen cell suspensions were ammonium-chloride-potassium lysis buffer-lysed, then washed with HBSS and resuspended in HNA.

Immunofluorescence and flow cytometry

We incubated 1×10^6 cells in 0.1 mL of HNA with 2.4G2 or human IgG for 15 minutes on ice, then stained with 1 μg each of fluorochrome-labeled mAbs specific for mouse IgE (clone RME-1), human IgE (clone MHE-18), or the anti-huFcεRIα mAbs AER-37 or 15.1, and/or mAbs to human c-Kit (clone 104D2), mouse c-Kit (clone 2B8) and/or human IL-3R (clone 6H6), mouse B220 (clone RA3-6B2) and/or human CD3 (clone OKT3), or mouse CD3 (clone 145-2C11) (all fluorochrome-labeled mAbs from

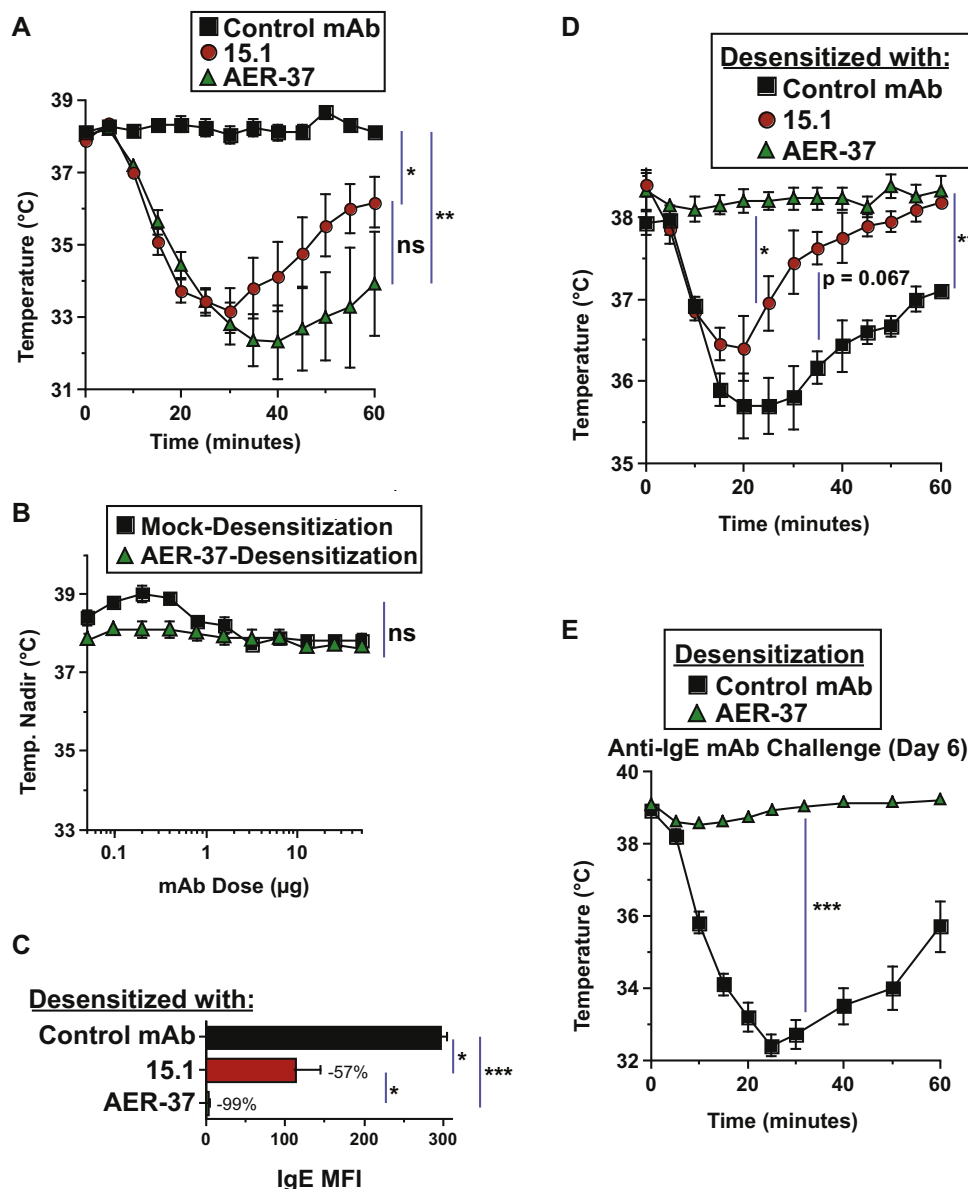


FIG 2. Desensitization of huFcεRIα mice with anti-huFcεRIα mAbs. **A**, huFcεRIα mice (3/group) were injected intravenously with 100 μg of mAb 15.1 or AER-37. Changes in rectal temperature were followed for 60 minutes. Means and SEMs are shown in this and in all other figures. **B**, huFcεRIα mice (5-6/group) were injected hourly intraperitoneally with doubling doses of AER-37 or an isotype-matched control mAb, starting with a dose of 97 ng and ending with a dose of 50 μg. The means and SEMs of the lowest rectal temperatures during the hour following each injection are shown. **C**, Peritoneal mast cells from mice (4/group) that were first injected on successive days with 8.125 μg doses of huIgE were mock-desensitized with an isotype control mAb or were desensitized with AER-37 or 15.1 as in **B** and were stained for huIgE and analyzed for membrane fluorescence by flow cytometry. **D**, huFcεRIα mice (4/group) that had been sensitized on successive days with 8.125 μg doses of huIgE and desensitized or mock-desensitized as in **B** were challenged intravenously with 100 μg of anti-human IgE mAb. Rectal temperatures were followed for the next 60 minutes. **E**, huFcεRIα mice (6-7 mice/group) that had been sensitized with human IgE and desensitized or mock-desensitized as in **B** were then injected every 3 days with 5 μg of mouse IgE and every 2 days with 50 μg of AER-37 or isotype control mAb. No temperature drops were seen with these repeated injections (not shown). Mice were challenged 6 days after desensitization with 100 μg of anti-human IgE mAb intravenously and rectal temperatures were followed for the next 60 minutes. Tests for statistical significance: **A**, 1-way ANOVA and THSDT; **B**, Mann-Whitney *U* test; **C**, 1-way ANOVA and THSDT; **D**, 1-way ANOVA and THSDT; **E**, Mann-Whitney *U* test. **P* < .05; ***P* < .01; ****P* < .001. MFI, Mean fluorescence intensity; ns, not significant.

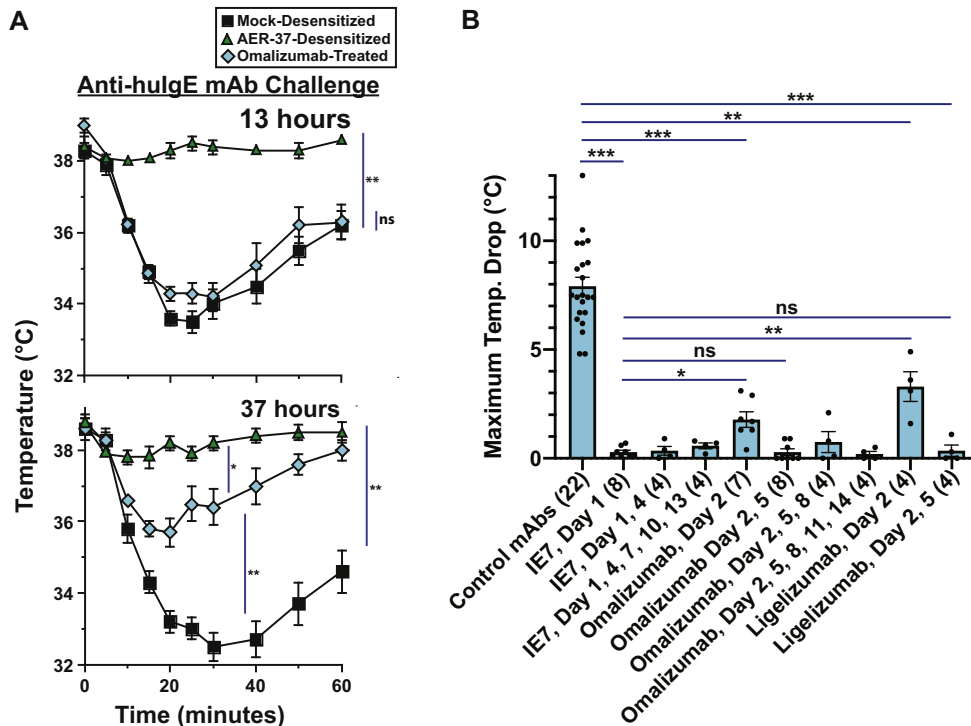


FIG 3. IgE-mediated anaphylaxis is suppressed more rapidly by desensitization with anti-huFcεRIα mAb than by omalizumab or ligelizumab. **A**, huFcεRIα mice (5–6/group) were injected intravenously on successive days with 10-μg doses of hulGE and mock-desensitized or desensitized with AER-37 as in Fig 2, B, or injected subcutaneously with 500 μg of omalizumab. Different groups of mice were challenged intravenously with 100 μg of anti-hulGE mAb 13 or 37 hours after completion of desensitization (23 or 47 hours after omalizumab injection) and rectal temperatures were followed for the next hour. To maintain hulGE levels, mice challenged 37 hours after completion of desensitization (47 hours after omalizumab injection) were injected with an additional 10 μg of hulGE 24 hours prior to anti-hulGE mAb challenge. Data were analyzed for statistical significance by 1-way ANOVA. **B**, huFcεRIα mice (4/group in each of 2 separate experiments) were all injected intraperitoneally with 10 μg of hulGE anti-TNP mAb on day 0, and with repeat injections of the same mAb with each dose of IE7, omalizumab, or control mAb, including 1 day prior to antigen challenge. Different groups of mice also were administered control mAb (normal human IgG or CBDA [mouse IgG₁]), omalizumab, ligelizumab, or IE7 on the days shown. Mice were challenged intravenously with 20 μg of TNP-BSA 1 day after the last injection of control mAb, IE7, or omalizumab, after which rectal temperatures were followed for 60 minutes. Mean greatest decreases in rectal temperature for individual mice are shown. Mice were injected intraperitoneally with albuterol, 2.5 mg/kg and triprolidine, 200 μg 30 and 60 minutes prior to the initial dose of IE7 or CBDA to prevent IE7-induced anaphylaxis. IE7 and its control mAb were injected subcutaneously hourly at doubling doses (25, 50, and 100 μg) for the initial day of administration, then 100 μg subcutaneously on additional days of administration. Omalizumab and ligelizumab were injected subcutaneously at a dose of 500 μg. All mice were injected intraperitoneally with IL-4C (1 μg IL-4 + 6 μg BVD4-1D11) 1 day prior to TNP-OVA challenge to increase the severity of anaphylaxis. Results from both experiments were pooled and treatment with all control mAbs was pooled because the temperature drops for mice treated with control mAbs were similar. Numbers in parentheses show number of mice per group. Data were tested for statistical significance with a one-way ANOVA and THSDT. An additional analysis with the Kruskal-Wallis test gave identical results. **P* < .05; ***P* < .01; ****P* < .001.

BioLegend). Cells were washed twice, fixed with 2% paraformaldehyde, and analyzed with a Becton-Dickinson LSR II Flow Cytometer (BD Biosciences, Franklin Lakes, NJ). Mast cells were identified as high forward and side scatter cells that expressed IgE, FcεRIα, and Kit. Basophils had lower side and forward scatter and expressed IL-3R, IgE, and FcεRIα, but lacked Kit.

Surface plasmon resonance

The binding of Fab fragments of anti-huFcεRIα mAbs to soluble huFcεRIα was analyzed by surface plasmon resonance (SPR) using a Biacore T200 (GE Healthcare Life Sciences). Carboxymethylated dextran-coated Series S CM5 sensor chips (GE Healthcare Life Sciences) were used for all experiments. Chips were activated with N-hydroxysuccinimide/1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (NHS/EDC) and 200 RU FcεRIα was immobilized as

described.^{29–31} Two-fold serial dilutions of anti-huFcεRIα mAb Fab fragments in PBS were injected at 30 μL/min for 120 seconds for kinetic analysis, followed by regeneration with 10 mmol glycine pH 1.7. All data were analyzed using a 1:1 kinetic model in Scrubber (BioLogic Software, Campbell, Australia). Bulk refractive index shifts were included for each concentration injected, and the AER-37 data were fitted with a mass transport term. *K_D* values were calculated from *k_{off}*/*k_{on}*.

Statistics

A 1-way ANOVA, a 2-tailed Mann-Whitney *U* test, a Kruskal-Wallis test, and Tukey honest significant difference test (THSDT) were used when appropriate, to test for statistical significance. The tests used are reported in figure legends. A *P* value of <.05 was considered significant. Figures note *P* values of borderline significance (.10 > *P* > .05).

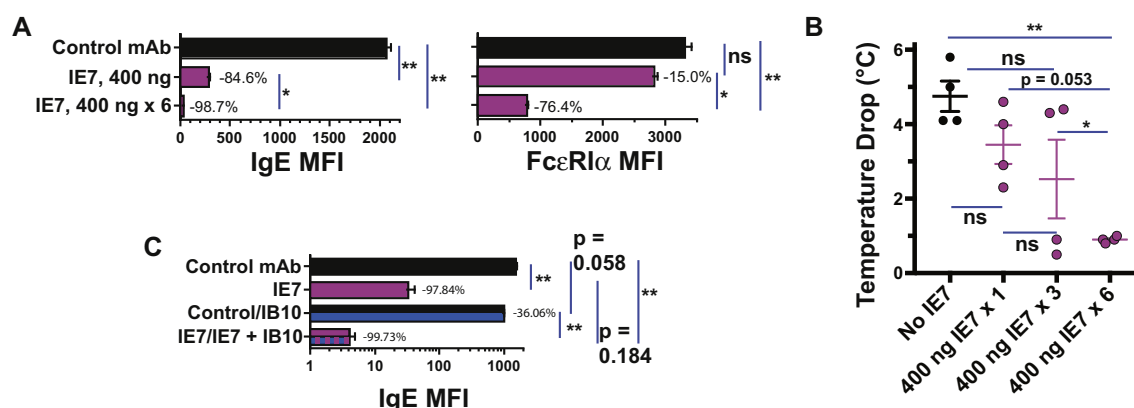


FIG 4. Repeated small doses of anti-huFcεRIα mAb can desensitize mice and remove membrane IgE and FcεRIα. **A and B**, huFcεRIα mice (4/group) received a single intraperitoneal 400-ng injection of mAb IE7, 3 hourly injections of 400 ng of IE7, or 6 hourly injections of an isotype control mAb. **A**, Six hours after the initial injection, mice were injected intravenously with 200 μg of IE7 and their rectal temperatures were followed for the next 60 minutes. **B**, Peritoneal mast cells from mock-desensitized mice or mice that were injected intraperitoneally once or every hour for 6 hours with 400 ng of IE7 were stained for mouse IgE and with mAb 15.1 for huFcεRIα 1 hour after the last injection of IE7 or control mAb. **C**, In a separate experiment, huFcεRIα mice (4/group) were sensitized with mouse IgE and injected intraperitoneally hourly for 8 hours with 400 ng of isotype control mAb (black bar), IE7 (red bar), with isotype control mAb for 8 hours plus IB10 for the last 4 hours (green bar) or with IE7 for 8 hours plus IB10 for the last 4 hours (blue bar). Peritoneal mast cells were stained for mouse IgE 1 hour after the last mAb dose. Tests used to determine statistical significance: **A**, 1 way ANOVA and THSDT; **B**, 1-way ANOVA and THSDT (without Tukey adjustment, differences between 1 and 3, 2 and 6, and 3 and 6 treatments are significant); **C**, 1-way ANOVA and THSDT (without Tukey adjustment, difference between control and IB10 is significant and for IE7 vs IE7 + IB10 $P = .072$). * $P < .05$; ** $P < .01$.

RESULTS

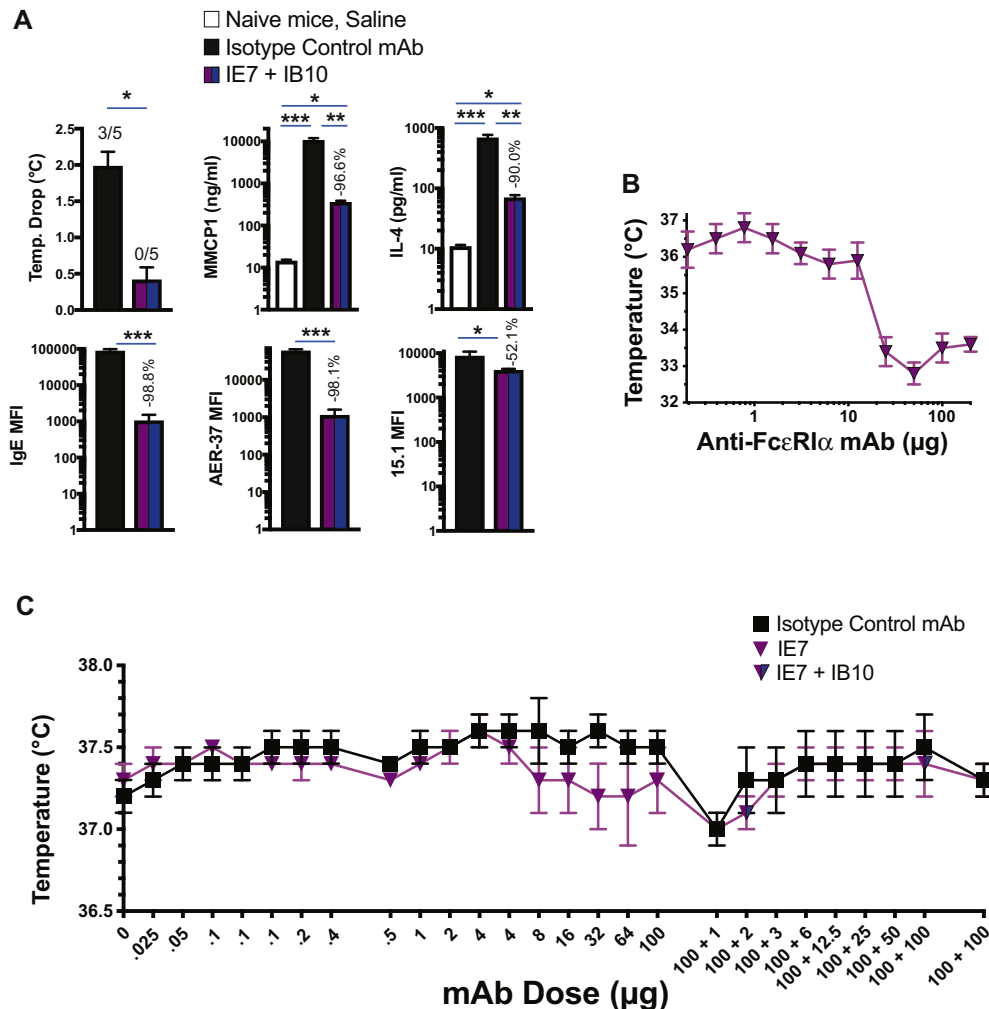
Determination of anti-huFcεRIα mAb affinity and avidity

Both ELISA and SPR were used to characterize the affinity and avidity of binding of the 4 anti-huFcεRIα mAbs used in this study. By ELISA, AER-37 and IE7 bound similarly to microtiter plate wells coated with huFcεRIα ectodomain, while the binding curves for 15.1 and IB10 were shifted to the right, but similar to each other (Fig 1, A). Additional ELISA-based studies focused on AER-37 and IE7, because our initial functional studies showed that they had greater ability than 15.1 or IB10 to rapidly suppress IgE-mediated anaphylaxis (see below). AER-37 bound better than IE7 to microtiter plates coated with decreased quantities of huFcεRIα ectodomain (Fig 1, B), which suggests that AER-37 has higher affinity than IE7 for this Ag. Consistent with this conclusion, AER-37 Fab was more potent than IE7 Fab at inhibiting the binding of biotin-labeled intact AER-37 and IE7 to wells coated with a suboptimal amount of huFcεRIα ectodomain (Fig 1, C). However, determination of AER-37 and IE7 affinity for huFcεRIα by SPR, the standard, commonly accepted way to measure a ligand's affinity for its target, suggests a more complex explanation for the differences in AER-37 and IE7 binding to huFcεRIα ectodomain. To perform the SPR studies, huFcεRIα ectodomain was immobilized on the SPR sensor chip and Fab fragments were flowed over at 30 μL/min for kinetic analysis. In contrast to the ELISA data, AER-37 and IE7 Fab fragments bound huFcεRIα ectodomain with similar affinities, with fitted K_D values of 518 nmol for mIE7 and 724 nmol for AER-37 (Fig 1, D and Table I). However, AER-37 Fab had an on-rate that was ~3-fold faster than that of IE7 Fab, and a similarly higher off-rate. This suggests that ELISA techniques that are

commonly used to qualitatively estimate affinity may be sensitive to the kinetics of binding in addition to the K_D . Attempts were also made to measure 15.1 and IB10 affinity by SPR, but the affinities of these mAb Fabs were too low to be accurately measured by our equipment.

huFcεRIα mice can be rapidly desensitized with anti-huFcεRIα mAbs

Initial experiments evaluated the ability of 2 anti-huFcεRIα mAbs to induce anaphylaxis when injected into huFcεRIα mice. Both mAb 15.1,²² a mouse IgG₁ that competes with IgE for binding to huFcεRIα, and mAb AER-37,²³ a mouse IgG_{2b} that binds huFcεRIα regardless of its association with IgE, induced hypothermia (evidence of shock) when injected intravenously at a dose of 100 μg (Fig 2, A). In contrast, neither mAb induced hypothermia when injected through a rapid desensitization approach, starting with a dose of 97 ng and doubling the dose hourly until a dose of 50 μg was reached (Fig 2, B, shown for AER-37 only). In a subsequent experiment in which huFcεRIα mice were sensitized twice with 8.125-μg doses of huIgE, then mock-desensitized with an isotype-matched control mAb or rapidly desensitized with AER-37 or 15.1, followed by intravenous challenge with anti-huIgE mAb, rapid desensitization with 15.1 removed 55% to 60% of IgE from mast cells and partially suppressed the development of hypothermia, while rapid desensitization with AER-37 removed 99% of IgE from mast cells and completely suppressed hypothermia (Fig 2, C and D). Repeated, single injections of AER-37 into AER-37 desensitized mice failed to induce hypothermia (not shown) and maintained suppression of IgE-mediated anaphylaxis for at least 6 days (Fig 2, E).



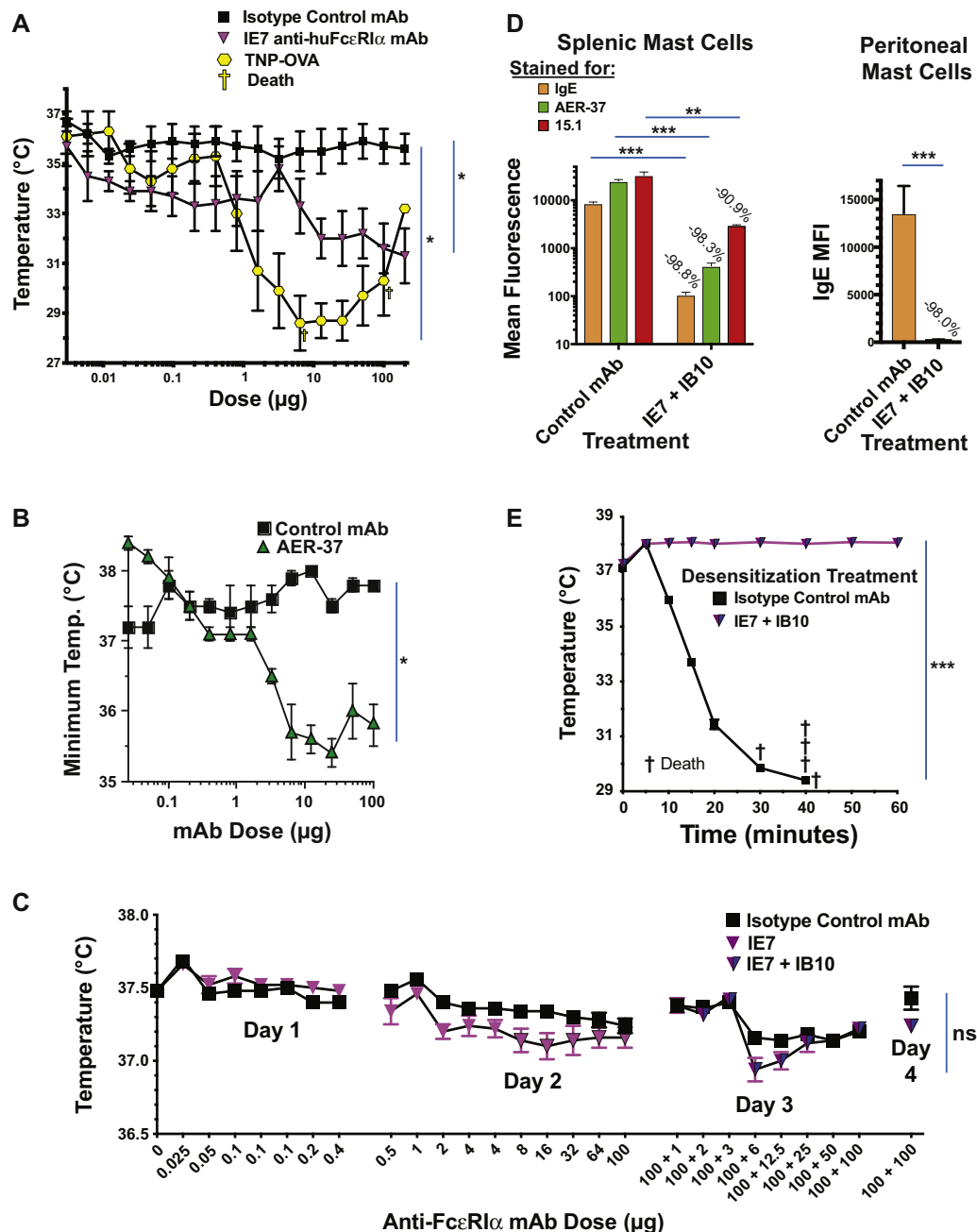


FIG 6. Rapid desensitization of reNSGS and reNRGS mice. **A**, reNSGS mice (4/group) were sensitized with mouse IgE anti-TNP mAb and mock-desensitized or desensitized by hourly intraperitoneal injections of doubling doses of IE7 or TNP-OVA. The lowest temperatures during the hour after each injection are shown. **B**, In a separate experiment, reNSGS mice (4/group) were sensitized with human IgE anti-TNP mAb and mock-desensitized or desensitized with doubling doses of AER-37. The lowest temperatures during the hour after each injection are shown. **C**, reNRGS mice (4-5/group) were sensitized with humanized IgE anti-TNP mAb and mock-desensitized or desensitized with the doses shown of IE7 plus IB10 over a 3-day period. Mice were observed for changes in rectal temperature and activity during desensitization; no changes in mouse activity were observed. For day 3 of desensitization, the first number refers to the dose of IE7, the second number to the dose of IB10 or isotype control mAb. **D**, Peritoneal human mast cells from the mock-desensitized or desensitized mice from **C** were stained for human IgE and splenic mast cells from these mice were stained for human IgE and with fluorochrome-labeled AER-37 or 15.1 and analyzed by flow cytometry for surface fluorescence. **E**, Desensitized and mock-desensitized mice from **C** were challenged intravenously with 1 μg of TNP-OVA and followed for the next 60 minutes for survival and rectal temperature. Tests used to determine statistical significance: **A**, 1-way ANOVA and THSDT; **B-E**, Mann-Whitney *U* test. **P* < .05; ***P* < .01; ****P* < .001.

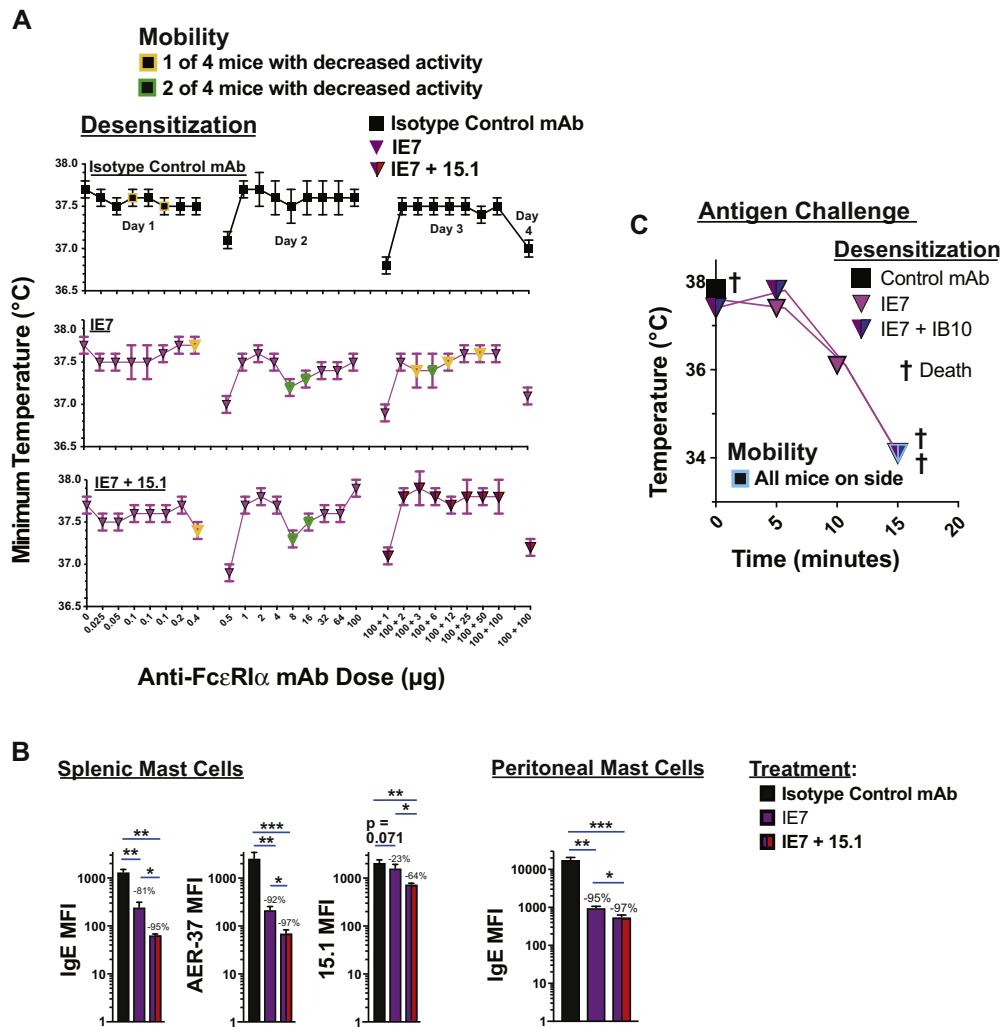


FIG 7. Rapid desensitization of mouse IgE-sensitized reNRGS mice. **A**, reNRGS mice (4/group) were sensitized with mouse IgE anti-TNP mAb and mock-desensitized or desensitized over a 3-day period with IE7 or IE7 plus 15.1, as indicated. Rectal temperature and activity levels were observed during the desensitization process. For day 3 of desensitization, the first number refers to the dose of IE7, the second number to the dose of 15.1 or isotype control mAb. **B**, Human splenic and peritoneal mast cells from desensitized and mock-desensitized mice were stained for mouse IgE. Human splenic mast cells from these mice were also stained with fluorochrome-labeled AER-37 and 15.1. Cells were analyzed for surface fluorescence by flow cytometry. **C**, One mouse from each group was challenged intravenously with 1 μg of TNP-OVA and survival and rectal temperatures and activity levels were followed for 20 minutes. Temperature data were analyzed for statistical significance by 1-way ANOVA and THSDT or Mann-Whitney *U* test. **P* < .05; ***P* < .01; ****P* < .001.

administration of 500 μg of omalizumab or the higher affinity anti-huIgE mAb, ligelizumab,³³ only partially suppressed IgE-mediated anaphylaxis in 48 hours, although suppression was nearly complete after 5 days of treatment and persisted for at least 14 days with additional 500-μg doses (Fig 3, C and D).

Repeated small doses of IE7 anti-huFcεRIα mAb desensitize huFcεRIα mice

The above-described studies showed that rapid desensitization of huFcεRIα mice with serially doubling doses of an anti-huFcεRIα mAb that does not compete with IgE can safely suppress IgE-mediated anaphylaxis. However, they left open the possibility that even small doses of this mAb could desensitize mice. To test this possibility, we treated huFcεRIα mice with 1, 3,

or 6 hourly 400-ng doses of the noncompeting anti-FcεRIα mAb, IE7, then challenged them with 200 μg of the same mAb. Even a single 400-ng dose of IE7 removed most IgE and some FcεRIα from mast cells (Fig 4, A) and tended to decrease the subsequent response to a 200-μg dose of the same mAb (Fig 4, B). Suppression of anaphylaxis remained incomplete when mice were treated with 3 hourly 400-ng doses of IE7 before the 200-μg challenge; however, treatment with 6 hourly 400-ng doses nearly completely blocked the response to challenge with 200 μg of IE7 and, in a separate experiment, removed >98% of IgE and 76% of FcεRIα from mast cells. In an additional experiment, 8 hourly 400-ng doses of IE7 removed 97.8% of IgE from sensitized mast cells; adding 400 ng of the competing anti-FcεRIα mAb IB10/h during the last 4 hours caused the loss of >99.7% of mast cell IgE, while 4 hourly doses of IB10 alone

caused the loss of only 36% of mast cell IgE (Fig 4, C). The difference between 97.8% inhibition and 99.7% inhibition was statistically significant when the 2 values were compared directly with a Mann-Whitney *U* test ($P = .0286$), but significance was lost when a Tukey correction for multiple comparisons was applied ($P = .184$, as shown in the figure). Taken together, these observations suggest that continuing exposure of mast cells from huFcεRIα mice to low concentrations of a noncompeting anti-huFcεRIα (1) modifies FcεRIα so that it has reduced ability to bind IgE and (2) makes mast cells at least partially anergic to further FcεRIα cross-linking, and that (3) treatment with a combination of a competing anti-FcεRIα mAb and a noncompeting anti-FcεRIα mAb may more completely remove IgE from its target than either mAb alone (the difference between IgE removal with IE7 vs IE7 plus IB10 did not quite reach significance [$P = .072$]). Two different mechanisms could explain this observation. First, 2 mAbs binding to different epitopes of the same cell surface molecule should enhance cross-linking of that molecule, which should increase its internalization. Second, one mAb (IE7) is more effective than the other (IB10) at dissociating IgE from FcεRI, while IB10 binding to FcεRI that has lost IgE then prevents FcεRI from rebinding IgE.

Rapid desensitization with anti-FcεRIα mAb suppresses anaphylaxis in actively sensitized huFcεRIα/F709 mice

Although passively sensitized huFcεRIα mice can develop anaphylaxis, the extent of hypothermia following antigen or anti-IgE mAb challenge can be less than that observed in wild-type (WT) BALB/c mice (not shown). In addition, although it is relatively easy to actively sensitize WT BALB/c mice to egg by immunizing them epicutaneously, then OG with a combination of EW plus EYP, similar treatment of huFcεRIα mice failed to sensitize them to develop anaphylaxis in response to intravenous or OG challenge with EW plus EYP (not shown). This may reflect decreased expression and/or decreased signaling efficiency of the chimeric FcεRI in huFcεRIα mice. For these reasons, we were concerned that the passively sensitized huFcεRIα model might be “too easy” to desensitize to reflect the challenges posed by this procedure in humans. To create a more challenging mouse model, we egg-immunized huFcεRIα/F709 mice, which, in addition to their expression of human, rather than mouse FcεRIα, express a mutant IL-4Rα chain (the signaling component of the IL-4 and IL-13 receptors³⁴) that transmits positive signals normally but lacks the inhibiting signaling component that is normally associated with this receptor.¹⁹ As a result, IL-4Rα/F709 mice are allergy prone.¹⁹ Indeed, huFcεRIα/F709 mice developed egg allergy when immunized epicutaneously, then OG with EW plus EYP, and developed anaphylaxis (hypothermia and, frequently, decreased mobility and a hunched appearance) when challenged intravenously with EW plus EYP, even though they were pretreated with anti-FcγRIIb/FcγRIII mAb (2.4G2) to block IgG-mediated anaphylaxis³⁵ (Fig 5, A, left upper panel, purple bar). In addition, mast cells in these immunized mice stained brightly for IgE and FcεRIα (Fig 5, A, lower panels, purple bars; compare this IgE staining to IgE staining of mast cells from huFcεRIα mice in Fig 4, A). Consistent with this, huFcεRIα/F709 mice were more difficult to safely rapidly desensitize than huFcεRIα mice were, in that the former developed sizeable temperature drops when inoculated hourly

intraperitoneally with doubling doses of IE7, particularly when the dose was increased from 12.5 to 25 μg (Fig 5, B). This led us to attempt a gentler, yet more rigorous 3-day desensitization procedure that treated mice with IB10 after initial IE7 treatment (Fig 5, C). This treatment safely removed >98% of IgE and >50% of FcεRIα that was associated with neither IgE nor IB10 from peritoneal mast cells (Fig 5, A, compare purple and cyan bars in the 3 lower panels) and protected mice from the anaphylactic response to intravenous EW plus EYP challenge, with no change in mobility, a temperature drop no larger than that observed in nonsensitized mice that were injected with egg (not shown) and 98.6% and 90% reductions in the MMCP-1 and IL-4 responses to egg challenge (Fig 5, A, compare purple and cyan bars in 3 upper panels).

In an additional, similar experiment, percentages of blood basophils and peritoneal mast cells following anti-FcεRIα desensitization or mock-desensitization were determined by immunofluorescence staining and flow cytometry. Results (see Fig E1 in this paper's Online Repository at www.jacionline.org) showed nearly complete loss of blood basophils, but no significant effect on the percentage of mast cells in peritoneal exudate. Consistent results were obtained in additional experiments.

Rapid desensitization safely suppresses IgE-mediated anaphylaxis in passively sensitized reNRGS mice

Although our studies with huFcεRIα/F709 mice extended our results to a model that may more closely resemble humans who are allergic than the huFcεRIα mouse model, it still studied mouse basophils and mast cells, which have important differences from the homologous human cell types.³⁶⁻³⁹ One important difference, based on *in vitro* studies, is that it has been more difficult to remove IgE and FcεRIα from the human than the mouse cells by treatment with antigen and anti-FcεRIα mAb.³⁹ Consequently, it was important to study rapid desensitization with anti-huFcεRIα mAbs in a mouse model that featured human mast cells and basophils. To do this, we used a model in which immunodeficient NSGS and NRGS mice, which express 3 transgenic human cytokines that promote mast cell development, survival, and activation, are reconstituted with T cell–depleted human cord blood.^{20,21} These mice, in addition to having mouse mast cells, develop large numbers of partially activated human mast cells in spleen, peritoneum, and other sites, and after passive sensitization with mouse IgE (which binds with high affinity to human FcεRI) or human IgE are considerably more sensitive to antigen challenge than are WT BALB/c mice. Because these reNSGS and reNRGS mice, in our hands, do not reliably generate antigen-specific IgE responses, we evaluated them after passive sensitization with mouse IgE anti-TNP mAb or chimeric IgE anti-TNP that had mouse V regions and human κ and ε chain C regions (referred to as huIgE anti-TNP). Rapid desensitization of 1 set of these IgE anti-TNP mAb-sensitized mice (several of which were hypothermic even before treatment) with IE7, using a protocol similar to that used with huFcεRIα mice, induced mild to moderate hypothermia; however, this was less severe than when the same mice were rapidly desensitized with the relevant antigen (Fig 6, A). Additional experiments confirmed the observation that AER-37 desensitization of reNSGS mice presensitized with huIgE anti-TNP mAb developed moderately severe hypothermia during desensitization when our standard

rapid desensitization protocol was used (Fig 6, B). This led us to develop a 3-day desensitization protocol that was based on the one used to desensitize huFcεRIα/F709 mice, starting with multiple small doses of IE7 and ending with large doses of both IE7 and IB10 (Fig 6, C). Using this protocol to desensitize reNRGS mice that had been sensitized with huIgE anti-TNP mAb (which binds to human, but not mouse FcεRI), we detected little or no hypothermia and no changes in mouse activity during desensitization (Fig 6, C). Furthermore, the treatment removed >98% of IgE from the human mast cells in these mice (Fig 6, D) and completely protected them from challenge with TNP-OVA, which killed all of the mock-desensitized mice within 40 minutes (Fig 6, E).

Rapid desensitization with anti-huFcεRIα mAbs does not prevent anaphylaxis in murine IgE-sensitized reNRGS mice

As a control to show that the desensitization procedure did not nonspecifically make reNRGS mice insensitive to antigen, we similarly attempted to desensitize reNRGS mice that had first been sensitized with mouse IgE anti-TNP mAb, which binds to both mouse and human FcεRI. Because NOD background, SCID, γ_c -deficient NSG mice that have not been reconstituted with human cells have mouse mast cells, they develop anaphylaxis if sensitized with mouse IgE anti-TNP mAb and challenged intravenously with TNP-OVA (see Fig E2 in this paper's Online Repository at www.jacionline.org); consistent with this, both mouse and huIgE anti-TNP-mAb sensitize huFcεRIα mice to develop anaphylaxis in response to TNP-OVA, while mouse, but not huIgE anti-TNP mAb sensitizes WT BALB/c mice (see Fig E3 in this paper's Online Repository at www.jacionline.org). In reNRGS mice that had been sensitized with mouse IgE anti-TNP mAb, treatment with increasing doses of IE7, followed by IE7 plus 15.1, removed mouse IgE anti-TNP mAb from human mast cells without significant changes in rectal temperature as compared to mock-desensitized mice, although 2 of 4 mice in each desensitized group demonstrated a temporary decrease in spontaneous activity (Fig 7, A and B). Note that the combination of mAbs IE7 and 15.1 removed significantly more IgE from splenic mast cells than did IE7 alone; for peritoneal mast cells, the difference was statistically significant ($P = .0286$) when the 2 relevant groups were compared directly with a Mann-Whitney U test, although significance was lost when Tukey correction for multiple comparisons was applied ($P = .069$, as shown in Fig 7, B).

Desensitization with IE7 with or without 15.1 should not affect mouse IgE anti-TNP mAb on mouse mast cells, because it is unable to bind to the mouse FcεRI on these cells. Consistent with this, desensitization with IE7/IB10 delayed, but does not prevent TNP-OVA-induced lethal anaphylaxis in mouse IgE anti-TNP mAb-sensitized mice (Fig 7, C). Note that the mock-desensitized and IE7-desensitized mice died so quickly that no changes in activity were observed, while the IE7 plus IB10-desensitized mouse survived long enough to show an inability to stand before dying.

DISCUSSION

The data described in this paper build on our previous study of rapid desensitization of BALB/c mice with an anti-mouse FcεRIα mAb¹¹ by showing the following: (1) A similar process can be

performed with anti-huFcεRIα mAbs in mice that have humanized or human mast cells that have been passively or actively sensitized with IgE. The procedure was both safe and effective in mice that expressed human instead of mouse FcεRIα, in mice that also expressed a mutant IL-4Rα that considerably increased their susceptibility to allergy, and in mice that developed large numbers of cytokine-activated human mast cells, although desensitization needed to be performed more slowly in the latter 2 strains to avoid inducing anaphylaxis. (2) Desensitization with a noncompeting mAb that binds huFcεRIα regardless of its association with IgE, desensitizes mice considerably more rapidly than does desensitization with a competing anti-huFcεRIα mAb, although present data do not indicate whether this results from differences in mAb competition with IgE or from differences in mAb affinity for FcεRI. (3) mAbs of the former type more completely remove IgE than huFcεRIα from mast cells. (4) A combination of a competing and a noncompeting anti-huFcεRIα mAbs appeared to be somewhat more effective at removing IgE from mast cells than a noncompeting mAb alone. And (5) noncompeting anti-huFcεRIα mAb acted more rapidly than the anti-IgE mAbs, omalizumab and ligelizumab, at removing IgE from mast cells. These observations have both basic and clinical implications.

Speed and safety of rapid desensitization with anti-FcεRIα mAbs

Individuals who are allergic produce increased quantities of IgE as well as cytokines that promote isotype switching to IgE (IL-4 and IL-13⁴⁰) and cytokines that promote mast cell and/or basophil development, proliferation, and activation (IL-3, GM-CSF, IL-9, SCF, and IL-33⁴¹⁻⁴⁵). Consequently, individuals who are allergic have increased numbers of mast cells and basophils that express increased FcεRI and IgE^{46,47} and have increased sensitivity to stimuli, such as IgE/FcεRI cross-linking, that induce degranulation.⁴⁸ In addition, target cells, such as smooth muscle, gut, and lung epithelium, and vascular endothelium in these individuals may have increased sensitivity to mast cell-released mediators.⁴⁹ These considerations would predict a greater likelihood of untoward reactions during rapid desensitization with either antigen or anti-FcεRIα mAbs; comparison of our results in hu FcεRIα mice versus hu FcεRIα/F709, reNSGS, and reNRGS mice demonstrates that this was indeed the case. Nevertheless, both the safety and efficacy of rapid desensitization with anti-FcεRIα mAb could be maintained by lengthening the rapid desensitization process, with incorporation of a more sustained period of exposure to doses of anti-FcεRIα mAbs that were too small to trigger a clinically apparent response but sufficient (based on *in vitro* studies^{38,50,51}) to partially desensitize mast cells and basophils, allowing the safe administration of the larger mAb doses required for full desensitization.

Modulation of IgE and FcεRI by anti-FcεRIα mAbs

One difference between our *in vivo* results and previous *in vitro* observations is the ease with which even small doses of anti-hu FcεRIα mAbs decreased expression of both humanized mouse and human mast cell FcεRIα and especially mast cell IgE expression *in vivo*, although *in vitro* studies with human mast cells that had been sensitized with IgE and treated with up to 2 μ g/mL

of anti-IgE mAb failed to show significant loss of mast cell IgE.³⁶ Although the loss of nearly all IgE by mast cells (and the depletion of basophils) in anti-huFcεRIα mAb-treated mice explains this mAb's ability to suppress IgE-mediated anaphylaxis, the mechanisms involved remain to be determined. IE7 and AER-37, which do not compete with IgE for binding to FcεRI, had a much greater ability to rapidly remove IgE from mast cells than mAbs that compete with IgE for binding, such as 15.1 and IB10, yet IE7 and AER-37 have considerably less ability to remove IgE from mast cells *in vitro* than *in vivo* (data not shown). Furthermore, IE7 and AER-37 are more effective at removing IgE from mast cells than at removing IgE-unbound FcεRI from the mast cell surface (as determined by staining with 15.1, which is not cross-blocked by IE7 or AER-37). The difference between IgE and FcεRI down-modulation was particularly marked when mice were treated for a relatively short time with relatively low doses of IE7. Further study is required to determine whether treatment with a noncompeting anti-FcεRIα mAb acts *in vivo* to directly facilitate the dissociation of IgE from FcεRIα without modifying FcεRIα structure, to change the structure of FcεRIα or its positioning on the cell membrane so that it has reduced affinity for IgE, or causes FcεRI internalization, followed by reexpression on the cell exterior with loss of IgE, or through another mechanism. Further study is also required to determine whether univalent forms of non-competing anti-FcεRIα mAbs, which should have increased safety because of their inability to directly cross-link FcεRI, can remove IgE from FcεRI, and how mAb on-rate, off-rate, and combined affinity for FcεRIα influence the efficacy and safety of mast cell/basophil desensitization.

Treatment with 1 versus 2 anti-huFcεRIα mAbs

Although mAbs that compete with IgE for binding to FcεRIα have limited ability to rapidly deplete IgE from sensitized mast cells, they appear to enhance IgE-depletion by noncompeting anti-huFcεRIα mAbs. A combination of the noncompeting mAb IE7, with the competing mAb 15.1, removed significantly more IgE from splenic mast cells than did IE7 alone (Fig 7, B). Removal of IgE from peritoneal mast cells by IE7 plus 15.1 appeared to be greater than removal by IE7 alone, although this difference was not statistically significant when *P* values were corrected for multiple comparisons (Fig 7, B). Similarly, the combination of IE7 and the competing mAb IB10 appeared to remove more IgE from peritoneal mast cells than did IE7 alone, but the result was again only significant if not corrected for multiple comparisons (Fig 4, C). To increase statistical power, we used a joint probability distribution analysis to combine the probabilities for the 2 comparisons of peritoneal mast cell IgE depletion with a noncompeting plus a competing anti-huFcεRIα mAb versus a noncompeting mAb alone; this analysis indicates that it is highly likely that addition of a competing mAb increases the ability of a noncompeting mAb to deplete mast cell IgE (*P* = .013).

The incremental absolute decrease in IgE when mice are injected with competing and noncompeting mAbs can be small (1%–2%; however, this additional decrease is considerable when evaluated as quantity of remaining IgE (ie, a decrease of 97.84% leaves 8-fold more IgE on mast cells than a decrease of 99.73% does). The additional IgE depletion when mice are treated with both mAbs may result from the competing mAb's ability to block reassociation of IgE with unbound cell membrane FcεRIα, through increased cross-linking (and internalization or

sequestration) of FcεRI when it is bound simultaneously by 2 mAbs that attach to different epitopes, or through both mechanisms. In our studies, it was necessary to treat mice with the noncompeting mAb prior to simultaneous treatment with both mAbs because the greater cross-linking of FcεRI by the combined mAb treatment induced severe anaphylaxis when initiated without prior desensitization with the noncompeting mAb alone. Further studies are required to determine whether the greater complexity and possible risk of treatment with 2 mAbs is justified by increased protection against anaphylaxis by the additional removal of cell-associated IgE.

Antibody versus antigen desensitization

Desensitization with anti-FcεRIα mAb instead of the sensitizing antigen offers several advantages: (1) it simultaneously blocks all IgE-mediated anaphylaxis, regardless of the antigen involved, while *in vivo* antigen desensitization is most typically Ag-specific;^{12,36,52} (2) the considerably longer *in vivo* half-life of IgG antibodies than most antigens^{53–55} maintains desensitization for a longer period of time without a requirement for additional doses of the desensitizing agent; and (3) desensitization with anti-FcεRIα mAb removes the complication of formation of immune complexes between a desensitizing antigen and preexisting Ag-specific IgG antibodies, including a boosting effect on production of such antibodies. The formation of such complexes may explain the relatively delayed response and greater toxicity of antigen than anti-FcεRIα mAb desensitization (Fig 6, A); initial small doses of the antigen may never reach the mast cell to desensitize it because they are neutralized by Ag-specific IgG antibody. It is only when such antibody is saturated by increasing doses of antigen that the antigen gains access to mast cell IgE; by that time, the increase in desensitizing antigen dose can be sufficient to induce anaphylaxis by triggering non-desensitized cells. This is not a problem for desensitization with anti-FcεRIα mAb. Partially offsetting these advantages, the cost of anti-FcεRIα mAb for drug desensitization or treatment of other IgE-mediated disorders is likely to be greater than the cost of desensitization with the relevant antigen, when antigen desensitization is feasible.

Advantages over anti-IgE mAbs

Anti-IgE mAbs, such as omalizumab, that have very limited ability to bind to FcεRI-associated IgE, but can neutralize free IgE, preventing its binding to FcεRI,^{47,56} have little ability to induce anaphylaxis⁵⁷ and have been shown to suppress some disorders that are partially IgE-mediated, such as asthma.^{56,58,59} These mAbs have also been shown to increase the quantity of an allergenic food that can be eaten by an individual who is food-allergic without developing disease^{60,61} and to suppress disease development during oral desensitization for food allergy.^{60,62} However, these mAbs have limitations that are not seen with anti-huFcεRIα mAbs: (1) because they have little ability to bind to cell-associated IgE, they take much longer than noncompeting anti-FcεRIα mAbs to remove it and probably never remove it as completely as anti-FcεRIα mAbs; (2) because they compete with IgE for binding to FcεRI, they lose effectiveness in individuals who have very high IgE levels⁶³—the individuals who are most likely to require treatment. This is much less of a problem for noncompeting anti-FcεRIα mAbs.

Comparative clinical trials between anti-IgE mAbs and rapid desensitization with anti-FcεRIα mAbs are required to determine whether these features make an important clinical difference.

In sum, our results show that rapid desensitization with anti-huFcεRIα mAbs is an effective way to polyclonally desensitize humanized and human mast cells, even in mice that resemble humans who are hyperallergic; furthermore, this procedure appears to be safer than the rapid desensitization with antigens that is commonly used to permit treatment with allergenic drugs. Optimization of this procedure and its testing in nonhuman primates and eventually, patients with allergy may provide a novel and powerful approach to ameliorate allergic disorders.

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Clinical implication: Rapid desensitization with anti-FcεRIα mAbs may be a safe and effective way to suppress human IgE-mediated allergy.

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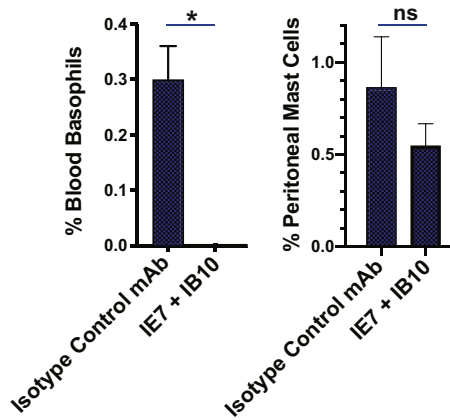


FIG E1. Effects of rapid desensitization on basophil and mast cell number. In an experiment similar to that shown in Fig 5, huFcεRIα/F709 mice that had been induced to develop egg allergy were desensitized over a 3-day period with IE7 followed by IE7 plus IB10 (see Fig 5 legend for details). Following desensitization or mock-desensitization, mice were bled and peritoneal lavage cells obtained. Cells were stained for kit, IL-3R, DX-5, and TCR-β and analyzed by flow cytometry. Basophils were identified as kit⁺ IL-3R⁺ DX-5⁺ TCR-β⁻ cells with forward and side scatter slightly greater than lymphocytes. Mast cells were identified as kit⁺ IL-3R⁺ TCR-β⁻ cells with high forward scatter and very high side scatter. There were 3 mice in the isotype control group and 6 in the IE7 plus IB10-treated group. Data were analyzed with a 2-tailed Mann-Whitney *U* test. Means and SEMs are shown. In additional experiments, complete or near-complete loss of basophils was observed within 4 hours of a single injection of a single anti-FcεRIα mAb, while the percentage of mast cells in peritoneal exudate showed no consistent trend toward an increase or decrease following anti-FcεRIα mAb treatment. **P* < .05.

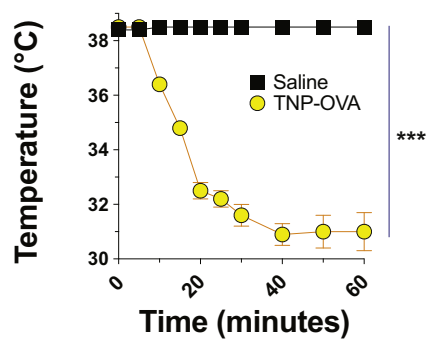


FIG E2. Unreconstituted NSG mice develop severe anaphylaxis when sensitized with mouse IgE anti-TNP mAb and challenged with TNP-OVA. NSG mice (4/group) were sensitized with mouse IgE anti-TNP mAb and challenged intravenously the next day with saline or TNP-OVA. Rectal temperatures were followed for the next hour. Data were analyzed for statistical significance by a 1-way ANOVA and THSDT. *** $P < .001$.

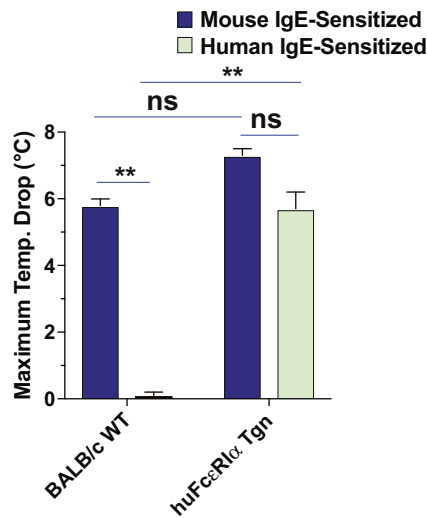


FIG E3. Cross-species sensitization of mouse and human mast cells. WT BALB/c and huFcεR1α mice (4/group) were sensitized with mouse IgE anti-TNP mAb or humanized IgE anti-TNP mAb and challenged intravenously the next day with TNP-OVA. Rectal temperatures were followed for the next hour. Data were analyzed for statistical significance by Mann-Whitney *U* test. ***P* < .01.