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Suppression of IgE B Cells and IgE Binding to FcεRI by Gene Therapy with Single-Chain Anti-IgE

Takayuki Ota,* Miyo Aoki-Ota,* Bao Hao Duong,**† and David Nemazee*‡

IgE plays a pivotal role in allergic reactions and asthma through its ability to bind to the mast cell FcRI for IgE (FcεRI). Current therapies to suppress such reactions include passive treatment with neutralizing Abs to IgE that block its binding to FcεRI. In theory, induction of immune tolerance in the B lymphocytes that carry IgE Ag receptors and give rise to IgE-secreting cells should provide longer term efficacy. However, recent data have suggested that such memory cells may lack cell surface IgE. Using a gene therapy approach, we show that a recombinant single-chain neutralizing anti-IgE could not only neutralize circulating IgE, but also reduce IgE+ B cell numbers and H chain transcripts. Therapeutic anti-IgE stimulated a calcium response in primary B cells or in a B cell line expressing membrane IgE and suppressed IgE secretion in vitro, suggesting that active signaling through membrane IgE likely promoted tolerance. Interestingly, upon subsequent challenge of anti-IgE-treated mice with an IgE cross-linking reagent capable of inducing activation of IgE-decorated mast cells, an anaphylaxis reaction was induced, apparently via membrane IgE likely promoted tolerance. Importantly, upon subsequent challenge of anti-IgE-treated mice with an IgE cross-linking reagent capable of inducing activation of IgE-decorated mast cells, an anaphylaxis reaction was induced, apparently via a FcγRIII pathway involving recognition of anti-IgE Ab itself. These studies have important implications for the optimal design of safe and effective anti-IgE therapies and suggest that the IgE memory B cells may be targeted by such genetic Ab therapies. The Journal of Immunology, 2009, 182: 8110 – 8117.

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**Abbreviations used in this paper: mIgE, membrane IgE; scFv, single-chain variable fragment; qPCR, quantitative PCR; TNP, 2,4,6-trinitrophenyl.

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cytometry.

To decrease the antigenicity of R1E4, rat-IgG1 was replaced with mouse IgG1 and several amino acid reverse mutations were introduced to L chain framework regions (supplemental Fig. 1A). To generate a secreted form in the final construct (pR1E4), the MHC transmembrane and cytoplasmic domains were excised. The specificity of recombinant single-chain membrane R1E4 to IgE was confirmed by the transient transfection to 293F cells and flow cytometry analysis of IgE binding (Fig. 1A). All of the plasmids used in in vivo injection were purified with an EndoFree Plasmid Maxi kit (Qiagen). For recombinant R1E4 (rR1E4), pR1E4 Ab-coding sequences were inserted into the pRRES-Zeocin hrGFP plasmid (pRRES-pR1E4), pRRES-pR1E4 (3 μg) was transfected into 293F cells with Lipo-fectamine2000 (Invitrogen) and stable cells were established. From this supernatant, rR1E4 was purified with a rProtein A column (GE Healthcare).

Quantitative PCR (qPCR)

Total RNA was purified from 2 to 3 million spleen cells of control or pR1E4-treated mice using a RNEasy Plus kit (Qiagen). Reverse transcription was performed with a QuantiTect Reverse Transcription Kit (Qiagen) per the manufacturer’s protocol. IgHe mRNA was quantitated using SYBR GreenER qPCR Supermix (Invitrogen) with 7900HT (Applied Biosystems) and normalized with CD19 mRNA. Oligonucleotide primers used for IgE detection were 5′-acactcggagatgcccagatc-3′ and 5′-ggcgtcactttgaagaatctcctg-3′ and for CD19 detection, 5′-aggtccattgcaggctagtt-3′ and 5′-gggctgctttgaagaaactctg-3′.

Flow cytometry analysis

Erythrocyte-depleted cells were suspended in ice-cold staining buffer (HBSS buffer including 0.5 mM EDTA, 0.05 mM sodium azide, and 0.5% BSA) with appropriately titrated Abs. The following Abs were used: CD45R/B220 (RA3-6B2; BD Biosciences) (Pacific Blue), FceRIα (MAR-1; eBioscience) (PE), IgE (23G3; eBioscience, or EM95) (FITC, PE, Alexa Fluor 647), CD49b (HMa2; BD Biosciences) (PE), allophycocyanin; ckit (2B8; eBioscience) (allophycocyanin; ckit), CD4 (GV1.5; BD Biosciences) (PerCP-Cy5.5), CD8 (53-6.7; BD Biosciences) (PerCP-Cy5.5), and strtrpavidin-PE-Cy7 (eBioscience). For intracellular IgE staining, cells were incubated during surface staining with unlabeled anti-IgE (EM95); after fixation and permeabilization using a Cytofix/Cytoperm kit (BD Biosciences), cells were stained with labeled EM95 conjugate. These Abs were purchased from eBioscience or BD Biosciences as indicated. Propidium iodide (Invitrogen) was included in some experiments to exclude dead cells. To calculate the total FceRI expression level on basophils based on IgE-binding capacity, FcεRs were preblocked with 2.4G2 for 10 min and the cells further incubated with purified IgE (IgELa) at 10 μg/ml for 30 min. Cells were then washed twice with FACS buffer and bound IgE was quantitated with anti-IgE conjugate. Data collection was done on an LSR II flow cytometer (BD Biosciences) and was analyzed using FlowJo software (Tree Star).

Hydrodynamic injection

Thirty micrograms of purified plasmid (pR1E4 or pUb control plasmid) was dissolved in 1.8 ml of TransIT-EE Delivery Solution (Mirus Bio) and injected via a tail vein. In the experiments depicted in Fig. 2, 10 μg of a second plasmid driving human placental secreted alkaline phosphatase (pLIVE-SEAP; Minus Bio) was coinjected, allowing one to monitor the efficiency of transfection by enzyme activity appearing in blood. All of the injected mice (five of control and eight of eight pR1E4 treated) analyzed on day 13 after plasmid injection were alkaline phosphatase positive (data not shown).

IgE-eliciting immunizations

OVA (Sigma-Aldrich) was prepared with alum (Inject; Pierce) at a ratio of 10 μg of protein:100 μg of alum/mouse and was given i.p. Goat anti-mouse IgD (0.2 ml; eBioscience) was injected i.p.

IgE ELISA

An IgE ELISA quantitation kit was purchased from Bethyl and used according to the kit instructions. In experiments involving pR1E4, purified EM95 (anti-IgE) was coated on Nunc Maxisorp plates and bound serum IgE was detected with HRP-conjugated goat anti-mouse IgE (Bethyl). The presence of rR1E4 did not interfere with the total IgE concentration measurement because R1E4 and EM95 see nonoverlapping epitopes; R1E4 blocks IgE binding to FcεR1, whereas EM95 cannot. For quantitation of free IgE, rR1E4 was coated on the plate and bound IgE was detected with HRP-conjugated goat anti-mouse IgE. Color was developed with UltraTMB (Pierce).

Cell culture

Splenocytes (2 × 10^7/ml) were cultured at 37°C/5% CO2 in a final volume of 2.5 ml with 25 ng/ml IL-4, 1 μg/ml anti-CD40 (1C10; eBioscience) with or without 20 μg/ml rR1E4 in Advanced RPMI 1640 medium supplemented with 5% FCS, 1× penicillin-streptomycin-glutamine (Life Technologies), 2 mM GlutaMAX-I (Life Technologies), and 55 μM 2-ME for 4 days. For Ca2+ mobilization analysis, splenocytes were cultured with 25 μg/ml IL-4 and 10 μg/ml anti-CD40. Culture supernatants were then tested for IgE concentration by ELISA; recovered cells were washed two times with FACS buffer before flow cytometry or Ca2+ mobilization analysis.

Calcium response

Cultured splenocytes were loaded with Fluor-4 (Invitrogen) per the manufacturer’s instructions. Calcium mobilization was induced by addition of 20 ng/ml Mabs at a cell concentration of 10^7/ml in volume of 0.5 ml. In some experiments, cells were preincubated with 10 μg/ml anti-mouse IgG1 Ab for 30 min before challenge and fluorescence analysis. Analysis was conducted using the FL1 channel of an LSR-II flow cytometer and analyzed with FlowJo software.

Anaphylaxis

One hundred micromgrams of EM95 was given per mouse retro-orbitally after anesthesia; rectal temperature was monitored with a RET-3 probe (Physiomet Instruments) for 60 min. In some experiments, 0.5 mg of 2,4,6-TNP (anti-FcyRI/II/III) Ab was given to each mouse 24 h before challenge. In the case of rR1E4 injection, 10 μg of 2,4,6-trinitrophenyl (TNP)-BSA and 100 μg of IgE anti-TNP (IgELa) were given per mouse and 50 μg of rR1E4 was given retro-orbitally; rectal temperature was monitored for 60 min.

### FIGURE 1. Generation and characterization of recombinant neutralizing anti-IgE single-chain Ab construct. A. The gene construct encoding rR1E4 (anti-IgE) showing intron/exon structure and selected features. Introns are depicted as thin lines. R1E4 scFv express under the control of human ubiquitin promoter. The R1E4 scFv was fused to hinge and F-encoding exons of the mouse IgG1 H chain. For membrane-bound anti-IgE expression, H-2Kb MHC class I transmembrane-coding sequences were included downstream. B. Control or recombinant plasmids expressing the membrane form of single-chain R1E4 were transiently cotransfected with enhanced GFP (EGFP) plasmid into 293 cells and the ability of the cells to bind to IgE was analyzed by flow cytometry.
Development of recombinant single-chain anti-IgE

In contrast to passive anti-IgE administration, anti-IgE delivery via gene therapy has the potential to provide permanent therapy of IgE-mediated disease. To study the effects of anti-IgE gene therapy in the mouse (an intensively studied model for human allergy, asthma, and anaphylaxis (13–15)), we generated a chimeric single-chain anti-IgE gene based on neutralizing rat anti-mouse IgE mAb R1E4 (9). R1E4, like omalizumab (Genetech) in the human system, is unable to activate mast cells via IgE bound to FcεRI, but binds free IgE. R1E4 Ig H and L chain variable region codons were cloned, sequenced, and joined together with a short linker sequence, yielding a scFv gene (Fig. 1A). The scFv gene was placed upstream of mouse IgG1 hinge and membrane proximal codons. Plasmids driving plasma membrane expression under the control of the human ubiquitin promoter (16) were prepared and validated (Fig. 1B). A modified plasmid, called pR1E4, encoding a secreted version of the rR1E4 chimeric protein (rR1E4) was generated. The biological effects of in vivo expression of plasmids encoding membrane or secreted proteins was tested by the hydrodynamic (naked DNA) injection method, which leads to transient expression in the liver. Because tolerance of cognate B cells to protein Ags can be induced either by membrane expression on the liver (17) or by soluble protein (18–20), we also compared in vivo efficacy of plasmids encoding membrane and secreted forms of recombinant anti-IgE.

Neutralizing serum IgE in vivo

pR1E4 plasmid given to BALB/c mice led to single-chain anti-IgE secretion lasting at least 30 days that diminished at later times (data not shown). At day 13 after pR1E4 injection, levels of free IgE were reduced to $1\%$ of those of control mice (Fig. 2A). There was also a marked decline in the levels of total IgE, which includes both free IgE and IgE-rR1E4 complexes (Fig. 2B). Plasmid encoding membrane-bound single-chain anti-IgE was also effective in reducing IgE levels, although the effect was less long-lasting (supplemental Fig. 2). For unknown reasons, plasmids encoding membrane-bound single-chain constructs were apparently somewhat toxic to expressing liver cells, even if they lacked specificity for IgE (data not shown). Therefore, additional experiments focused...
on the effects of the soluble form. Importantly, RNA analysis of the spleens of mice treated with pR1E4 revealed a >99% reduction in the levels of IgH mRNA (Fig. 2C). Consistent with the reduced IgE levels in pR1E4-treated mice, c-kit$^{+}$ peritoneal mast cells and CD49b$^{+}$ basophils in the bone marrow and spleens of anti-IgE-treated mice lacked detectable surface IgE, indicating that their FceRs had lost bound IgE (Fig. 2, D–H). Basophils were still present in these tissues as indicated by FcεRIα/CD49b double staining (Fig. 2G). In treated mice, FcεRI expression levels in basophils appeared higher than in control plasmid-treated mice, however, total IgE-binding capacity of basophils was somewhat reduced (supplemental Fig. 3, A and B). This discrepancy was probably because anti-FcεRI mAb MAR-1 binds more tightly to FcεRI lacking bound IgE.

**FIGURE 3.** Effects of pR1E4 plasmid treatment on ongoing IgE responses induced by OVA/alum immunization or goat anti-mouse IgD treatment. Two-month-old BALB/c mice injected i.p. 10 days previously (day 10) with either 10$^{10}$ g of OVA/alum or 200 l of goat anti-mouse IgD serum were treated with control or pR1E4 plasmids i.v. on day 0 and analyzed on days 0 and 12 for suppression of the IgE response. A, Total serum IgE levels on day 0 (f) and day 12 (□). B, Splenic IgHe mRNA levels assessed by qPCR. C–G, Flow cytometry analysis of IgE$^{+}$ B cells in spleens of mice injected with anti-IgD and subsequently treated with control or pR1E4 plasmid. C, Analysis of the frequencies of B220$^{hi}$ and B220$^{lo}$IgE$^{+}$ B cells among CD4$^{+}$CD8$^{+}$c-kit$^{+}$CD49b$^{+}$ gated viable spleen cells. D, Comparison of CD138 and IgD expression of B220$^{lo}$ and B220$^{hi}$ populations in control plasmid and pR1E4-treated mice, as gated in C. Left, B220$^{low}$ cells from control plasmid-treated mouse; center, B220$^{high}$ cells from control plasmid-treated mouse; and right, B220$^{high}$ cells of pR1E4-treated mouse. E, Analysis of treated and control anti-IgD-injected mice for the presence of cells that expressed high levels of intracellular IgE. F, Summary of pR1E4-induced reductions in IgE$^{+}$B220$^{high}$ and IgE$^{+}$B220$^{low}$ subsets using the analysis in C, G. Quantification of pR1E4-induced reduction in surface B220$^{high}$IgE$^{+}$ IgD$^{−}$ B cells as in D. Significance was calculated using Student’s t test. *, p < 0.01 and #, p < 0.05.

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* Mast cells and basophils were identified using flow cytometry 13 days after treatment, as described in Fig. 2.
* Control plasmid injection (n = 5 mice).
* Experimental plasmid injection (n = 8 mice).
$^a$ Statistically significant difference from control, p < 0.05, by two-tailed Student’s t test.

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than to FceRI carrying IgE (supplemental Fig. 3C). Reduced levels of bioactive IgE in pR1E4-treated mice did not lower the percentages or absolute numbers of mast cells and basophils (Table I). Overall, these data support the conclusions that 1) gene therapy with recombinant single-chain anti-IgE is feasible and can effectively neutralize circulating IgE; 2) reduced levels of bioactive IgE have surprisingly minimal negative effects on mast cell and basophil survival, at least at the time point tested, and 3) recombinant single-chain anti-IgE treatment could actively suppress new IgE production by B cells.

Recombinant anti-IgE treatment of preimmunized mice

We next determined whether pR1E4 could be used to treat and suppress ongoing, established IgE responses. Basal serum IgE levels in mice were quite low (~50 ng/ml in BALB/c mice and under 100 ng/ml in C57BL/6 mice). To evaluate the anti-IgE treatment, we used two different model responses: OVA immunization with alum adjuvant (OVA/alum), which is commonly used to elicit an Ag-specific IgE response, and anti-IgD treatment, a nonspecific B cell activator which elicits a stronger, but polyclonal IgE response, and anti-IgD treatment, a nonspecific B cell activator which elicits a stronger, but polyclonal IgE response. The low-affinity IgE receptor CD23 (23) on follicular B cells by subsequent in vivo expression of pR1E4 (Fig. 3A). However, IgHe mRNA qPCR analysis revealed that mice given pR1E4 after anti-IgD treatment still had high levels 12 days later, suggesting that IgE mRNA-producing cells remained in the spleen (Fig. 3B).

To identify the source of the IgE mRNA, we analyzed splenocytes from anti-IgD-treated mice for the presence of mIgE+B cells (Fig. 3, C–G). In control plasmid-treated mice, B220<sup>high</sup> and B220<sup>low</sup>IgE<sup>+</sup> B cells were detected (Fig. 3C). B220<sup>high</sup>B cells were mostly IgD<sup>+</sup>CD138<sup>+</sup> cells and possibly represented background staining of naive B cells (Fig. 3D, center). B220<sup>low</sup>B cells were mostly IgD<sup>+</sup>CD138<sup>–</sup>cells, indicating that they were IgE preplasma cells or B220<sup>low</sup>memory B cells (Fig. 3D, left). In pR1E4-treated mice, B220<sup>high</sup>IgE<sup>+</sup> B cells were detected, but most B220<sup>low</sup>IgE<sup>+</sup> B cells were lost. The remaining B220<sup>high</sup>IgE<sup>+</sup> B cells in pR1E4-treated mice were IgD<sup>+</sup>CD138<sup>+</sup> cells (Fig. 3D), suggesting that pR1E4 blocked development of IgE memory B cells and preplasma cells. The frequency of the
B220
low
IgE
+ fraction declined in pR1E4-treated mice (p = 0.0024; Fig. 3F). Intracellular staining for IgE (preblocked for surface staining) was conducted, revealing that plasma cells remained in the spleens of pR1E4-treated mice (Fig. 3E). Total numbers of B220
low
intracellular IgE
+ cells were on average lower in pR1E4-treated mice compared with control, but not to a statistically significant extent. We conclude that in anti-IgD-stimulated mice pR1E4 could neutralize circulating IgE and suppress the numbers of mIgE
+ B cells, but could not suppress fully developed IgE plasma cells.

Anti-IgE can block IgE secretion in vitro

To determine mechanistically how soluble rR1E4 affects IgE B cells, we assessed the effects of R1E4 mAb on cultured naive B cells induced to switch to IgE by the addition of IL-4 and anti-CD40. Although the binding of R1E4 has been mapped to the CH3 domain (9), it was not clear whether this epitope would be accessible on mIgE
+ B cells because on B cells mIg is oriented differently than in solution and is associated with Igα/β signal transducers. We found that mIgE
+ B cells could be clearly identified with both R1E4 IgG and rR1E4 Ab (Fig. 4A). Moreover, inclusion of rR1E4 both inhibited the secretion of IgE in the supernatant and also reduced the frequency of CD138
+ and CD138
+ IgE
+ B cells emerging in the cultures (Fig. 4, B–D). Ca
2+
+ signaling analysis indicated that rR1E4 Ab could induce Ca
2+
+ mobilization in mIgE
+ B cells upon supercross-linking by a second Ab (rat anti-mouse IgG1; Fig. 4E). These data indicate that anti-IgE can block the development of IgE plasma cells in vitro and may in part explain the in vivo effects of this treatment.

Anaphylaxis reaction

The ability of in vivo pR1E4 treatment to suppress allergic responses was tested by challenging mice on day 13 after treatment with an activating anti-IgE, EM95, which has the ability to induce mast cell degranulation and anaphylactic manifestations.Surprisingly, and notwithstanding their suppressed IgE levels, pR1E4-treated mice challenged in this way showed a systemic anaphylaxis reaction as indicated by rapidly lowered body temperature (Fig. 5A). Recently, an alternative pathway for anaphylaxis has been elucidated in which IgG1-immune complexes activate FcγRIII on basophils, releasing platelet-activating factor (15, 24–27). To test the possibility that this pathway was triggered by clustering of anti-IgE Abs upon challenge, pR1E4-treated or control mice were given FcγRII/III-blocking mAb 2.4G2 (28) 1 day before challenge. Indeed, anaphylaxis was blocked in pR1E4-treated but not in control mice receiving 2.4G2 (Fig. 5B). To test whether mouse IgG1 R1E4 could induce anaphylaxis directly in a FcεRI-independent manner, we preincubated TNP-BSA and IgE anti-TNP into FcεRIα
−/− mice and challenged them 1 h later with rR1E4. As shown in Fig. 5C, mice that received rR1E4 manifested an anaphylaxis reaction, indicating that rR1E4 itself triggered the reaction through FcγRs. Collectively, these data indicate that pR1E4...
treatment suppresses FcεRI-mediated anaphylaxis, but permits and in fact promotes FcγRIII-mediated anaphylaxis.

Discussion

Although it has been known that anti-IgE Abs that block IgE-FcεRI interactions prevent acute allergic reactions, it has not been clear that such Abs have strong effects on mIgE$^+$ B cells nor that a gene therapy approach to their application would be possible. Our data support earlier work of Habu and Nissonoff (6) who demonstrated that high doses of syngeneic anti-IgE could reduce IgE Ab-forming responses when given just before immunization. We show here that expression of an anti-IgE single-chain fusion construct can drive prolonged expression of a presumably dimeric anti-IgE protein in vivo and that this recombinant anti-IgE can block IgE binding to mast cells and basophils and suppress new IgE production. Because rR1E4 Ab was able to bind to mIgE$^+$ B cells in vitro and to trigger an altered in vitro response of these cells, we conclude that it can trigger signals in mIgE$^+$ B cells. Omalizumab (Xolair) neutralizes circulating IgE in humans, providing relief from allergic symptoms, apparently without reducing the underlying IgE stimulus. In a human trial of Xolair, the serum concentration of free IgE dropped rapidly (to 13.9 ng/ml), whereas the total IgE concentration increased over time (to $>$1000 ng/ml for 120 days) (29). By contrast, in the present study pR1E4 treatment suppressed both free IgE and total IgE and RNA analysis indicated that it also suppressed new IgE synthesis. These results may suggest that Xolair fails to suppress mIgE$^+$ B cells. If this is the case, our data suggest the possibility that more effective anti-IgE mAbs may be found with the potential for longer-term benefit. Such Abs would ideally share Xolair’s ability to block IgE-FcεRI interactions, but also be able to suppress IgE$^+$ B cells. Our preliminary studies indicate that Xolair is able to trigger Ca$^{2+}$ mobilization in a B cell line carrying human IgE. However, it may be that Xolair promotes rather than inhibits IgE production because of the quality of this signal or through its ability to generate higher-order immune complexes.

We presume that in vivo expression of rR1E4 mediates negative regulation of developing memory IgE B cells, probably by induction of apoptosis. However, the numbers of mIgE$^+$ B cells were too low to directly demonstrate this. Indeed, there is some controversy over whether mIgE$^+$ B cells stably exist in vivo (5). Several studies demonstrate that IgE class switching often occurs through an IgG1 intermediate (30–33). On the other hand, there are clear indications that B cells giving rise to IgE responses must carry mIgE and functionally signal through mIgE, at least for a short time. The membrane exons and protein sequences of mammalian Igs are well conserved and similar to other membrane Igs (34). Moreover, mutations or truncations of the membrane exons of IgE severely inhibit IgE responses, particularly secondary responses (7). The membrane form of ε chain mRNA may be poorly expressed owing to inefficient polyadenylation signals (35), suggesting that IgE memory B cells may express relatively low mIgE$^+$ levels. Nevertheless, Abs directed to the membrane form of IgE can have tolerogenic effects (8). In the present studies, we detected mIgE$^+$ cells and showed that their numbers increased in appropriately immunized mice. Moreover, mIgE$^+$ cells were specifically reduced in pR1E4-treated mice (Fig. 3C), indicating that negative regulation of developing IgE B cells by IgE-reactive ligands is possible.

The potency of IgE in allergic reactions is a result of the extraordinarily high affinity of FcεRI for monomeric IgE ($K_d = 10^{10}$ M$^{-1}$) combined with the powerful biological consequences of FcεRI ligation on mast cells and basophils (2). But the levels of IgE in blood were very low compared with other types of Igs, facilitating the effectiveness of treatment with passively administered IgE-neutralizing Abs and, as we show here, gene therapy using recombinant anti-IgE plasmid. We expressed the anti-IgE-neutralizing Ab as scFv fusion protein with a naked DNA injection method. The merit of this method is that gene expression is potentially long-lasting and it also permits the expression of membrane-bound proteins in vivo. Importantly, soluble rR1E4 expression lasted $>$3 wk after plasmid injection and could completely neutralize serum IgE, as measured by the level of free IgE and the levels of IgE bound to mast cells and basophils. Although convenient for proof of principle, plasmid injection provides only transient expression. Other modes of gene transfer, including retroviral transduction, should ultimately be more effective and practical in a clinical setting and may provide permanent IgE suppression. Long-term anti-IgE gene expression is predicted to not only suppress new plasma cell formation but also to neutralize IgE secreted by long-lived plasma cells that are no longer subject to regulation by surface Ig.

An unexpected finding was that even when IgE was neutralized mice still underwent anaphylaxis upon challenge with an activating anti-IgE stimulus, presumably through a FcγRIII-mediated pathway triggered by cross-linking IgE-rR1E4 complexes. We also showed that rR1E4 administration induced anaphylaxis in mice with IgE-Ag complexes. A comparable reaction may occur in a small fraction of anti-IgE-treated patients. According to the U.S. Food and Drug Administration (http://www.fda.gov/Cder/drug/InfoSheets/HCP/omalizumabHCP.htm), the frequency of anaphylaxis attributed to Xolair use in patients was $\geq0.2\%$. Of reported cases, 39% occurred after the first dose of Xolair, while only 19% occurred with the second dose, indicating the cause of anaphylaxis was not anti-Id (anti-Xolair) Ab. Xolair is a humanized IgG1 mAb that makes complexes with IgE at various ratios, the dominant complex being a trimer (36). If patients have both IgE and IgG Abs against the same Ag or IgE-bound foreign Ag itself, Xolair infusion may form higher order immune complexes and induce anaphylaxis through clustering of FcγRIII (Fig. 6). Conceivably, such reactions may be influenced by polymorphisms in FcγRIII and FcεRI (37, 38). To prevent anaphylaxis, mutagenesis of therapeutically anti-IgE to prevent FcγRIII binding may be desirable. Alternatively, expression of soluble monovalent or membrane-tethered anti-IgE may be used. Overall, our data suggest several ways that anti-IgE therapy can be improved to facilitate safety and longer-term effectiveness. We also show the feasibility of providing the Ab by gene therapy, which may provide a strategy to permanently suppress IgE reactions.

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Disclosures

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