“Accentuate the negative, eliminate the positive”: Engineering allergy therapeutics to block allergic reactivity through negative signaling

By: Andrew Saxon, Chris Kepley, Ke Zhang

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Abstract:

By targeting the dominant-negative signaling receptor FcγRIIb expressed on proallergic cells, we have developed 2 novel platforms for the treatment of IgE-mediated allergic disease. First is a genetically engineered bifunctional human fusion protein GE2, which is comprised of the Fc portions of human IgE and IgG1 with an interposed flexible linker designed as a long-term parenteral allergen-nonspecific therapy. GE2 blocks the effector phase of the IgE response in vitro in mice and human subjects and in vivo in the skin and airway and systemically in mice and monkeys. Whether reactivity against human GE2 in human subjects will limit its applicability remains to be determined. The second platform is designed to provide a safer form of allergen-specific immunotherapy and consists of genetically engineered chimeric human Fcγ-allergen proteins, with Fcγ–Fel d 1 as the prototype. The allergen portion binds to specific IgE on FcεRs, whereas the Fcγ portion coaggregates inhibitory FcγRIIb and drives inhibition of allergic reactivity. Fcγ–Fel d 1 blocked human mast cell Fel d 1–induced allergic reactivity in vitro and in vivo in murine models while functioning as an immunogen but not as an allergen.

Keyword: IgE therapeutics | immune response modifiers | allergy therapy | FcγRII | FcεRI | mast cells | basophils

Article:

Abbreviations used

AHR Airway hyperresponsiveness
BMMC Bone marrow–derived mast cell
DNP Dinitrophenol
ERK Extracellular signal–regulated kinase
GFD Chimeric protein composed of the human
Fcγ1 (γHinge-CHγ2-CHγ3), a flexible linker, and the major cat allergen (Fel d 1)
hGE2 Human bifunctional fusion protein consisting of part of the human
Fcγ1 (γHinge-CHγ2-CHγ3), a flexible linker, and part of the human Fcε(CHε2-CHε3-CHε4)

mGE2 Murine bifunctional fusion protein consisting of part of the murine Fcγ2a (γHinge-CHγ2-CHγ3), a flexible linker, and part of the mouse Fcε(CHε2-CHε3-CHε4)
PCA Passive cutaneous anaphylaxis
Syk Spleen tyrosine kinase

With respect to Johnny Mercer and Harold Arlen, who wrote "You've got to accentuate the positive, eliminate the negative,"[1] recognition that, at a molecular level, negative signaling can override positive signaling is a development with broad implications for both our understanding of immune homeostasis and for the development of immune-based therapies. Immunologists generally focus on "the positive," activation and development of a response, only to later recognize that negative regulatory processes function as the critical controls. Thus after decades of progress dissecting mast cell and basophil activation and mediator release, Daëron et al[2] were the first to demonstrate that FcεRI activation of mast cells could be downregulated by the inhibitory FcγRs and thereby provided the conceptual basis to translate this information into the therapeutic arena.

Targeting these inhibitory pathways, we have developed 2 distinct platforms for the potential treatment of human IgE-mediated disease (Table I). One platform uses negative signaling to drive non–antigen-specific suppression of allergic reactivity and is designed as a long-term treatment for any allergic disease, including severe food allergy (Fig 1, left panel).[3] The other platform uses the same negative signaling but to acutely block allergen-specific reactivity so that antigen-specific immunotherapy can be administered with greater safety as a disease-remitting intervention (Fig 1, right panel).[4] Thus although analogous in making use of FcγRII signaling, these 2 platforms are quite distinct.

Table I. Effects of hGE2 and GFD on allergic reactivity

Effect	hGE2	GFD
Inhibits signaling events through Syk-associated degranulation	Yes	Yes
Inhibits *in vitro* mediator release from human basophils and cultured cord blood–derived human mast cells	Yes	Yes
Inhibits human basophil and mast cell production of proinflammatory cytokines	Yes	Yes
Inhibits anti-IgE–driven mediator release from fresh lung tissue	Yes	Not tested
Inhibits FcεRI Langerhans cell IL-16 production	Yes	Not tested
Inhibits human B-cell IgE production *in vitro*	Yes	Not tested
Blocks PCA in FcεRI-transgenic mice to all allergens	Yes	No
Inhibits Fel d 1–mediated allergic degranulation in an antigen-specific manner	No	Yes
Blocks allergic responses in a mouse model of skin, lung, and systemic allergic reactivity	Yes	Yes
Fails to induce local or systemic reactivity on administration to Fel d 1–sensitized animals	Yes	Yes
Blocks Fel d 1–induced allergic reactivity when administered in a rush immunotherapy protocol	Not tested	yes
Immunotherapy modulates the antibody response to Fel d 1	No	Yes
Designed to work as ongoing long-term treatment	Yes	No
Designed to work as immunotherapy with long-term remission after stopping the agent	No	Yes

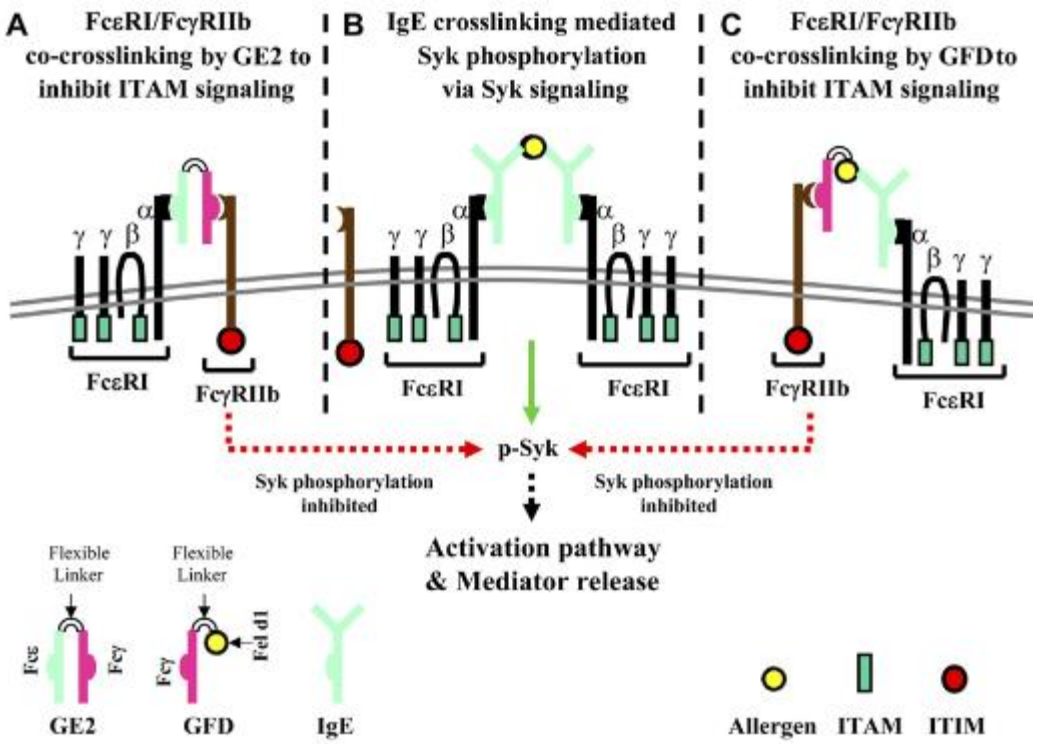

FIG 1. Action mode of the genetically engineered GE2 and GFD. Antigen cross-linking of 2 FceRIs andinduction of activation with enhancement of Syk and other downstream signaling molecules arediagrammed in themiddle panel, where FcgRII is unoccupied. GE2 co–cross-linking FcgRIIb to FceRI, whichresults in the inhibition of signaling events downstream of the FceRI immunoreceptor tyrosine–based acti-vation motif(ITAM), is shown in theleft panel, and the cross-linking mediated by GFD is presented in theright panel. A similar mode of action has been shown for mouse GE2.16ITIM, Immunoreceptor tyrosine–based inhibitory motif.

Negative signaling in human mast cells and basophils

The general concept of inhibitory signaling was recently summarized in the Journal.[5] After the activation of cells with the resulting phosphorylation of the immunoreceptor tyrosine–based activation motifs on activating receptors, negative inhibitory receptors containing immunoreceptor tyrosine–based inhibitory motifs recruit phosphatases that then dephosphorylate the activating receptors and turn the response off.[6] The key negative inhibitory receptor in the human allergic cascade is FcγRIIb.[7]

The cross-linking of FcεRI activates tyrosine phosphorylation of immunoreceptor tyrosine–based activation motifs in the β and γ FcεRI subunits in the cytoplasmic tails and leads to cell activation and degranulation in basophils and mast cells (Fig 1, middle panel).[8, 9] Tyrosine phosphorylation is a key event connecting FcεRI cross-linking to downstream signaling in human mast cells and basophils. Previous investigations have shown that the mitogen-activated protein kinases extracellular signal–regulated kinase (ERK) 1/2 and spleen tyrosine kinase (Syk) are quickly phosphorylated in IgE-stimulated human FcεRI-positive cells.[10] This leads to the classic immediate hypersensitivity reaction. Such an activation signal is balanced by the

inhibitory receptors on these cells. Human mast cells and basophils express FcγRIIb, which contains an immunoreceptor tyrosine–based inhibitory motif within its cytoplasmic tail.[11]

Using an antigen-nonspecific human bifunctional Fcγ-Fcε fusion protein (ie, human bifunctional fusion protein consisting of part of the human Fcγ1 [γHinge-CHγ2-CHγ3], a flexible linker, and part of the human Fcε[CHε2-CHε3-CHε4] or hGE2), we showed that coaggregation of FcεRI with FcγRIIb blocked in vitro and in vivo human basophil and mast cell function[3, 12, 13, 14, 15] through the reduction in the tyrosine phosphorylation of Syk, ERK, and several other cellular substrates and increased tyrosine phosphorylation of the adapter protein downstream of kinase, growth factor receptor–bound protein 2, and Src homology domain 2–containing inositol 5-phosphatase (Fig 1).[12, 13] Recent studies by Mertsching et al[16] using the mouse GE2 (murine bifunctional fusion protein consisting of part of the murine Fcγ2a [γHinge-CHγ2-CHγ3], a flexible linker, and part of the mouse Fcε[CHε2-CHε3-CHε4] or mGE2) confirmed and extended these studies. They showed that mGE2 decreased Syk and ERK 1 and 2 phosphorylation while inducing FcγRIIb phosphorylation and subsequent recruitment of Src homology domain 2–containing inositol 5-phosphatase 1 and Src homology domain 2–containing protein tyrosine phosphatase 1 and 2. These events were not observed in mast cells derived from FcγRIIb-deficient mice, proving the requirement for FcγRIIb binding in the inhibitory activity.

To determine whether our antigen-specific platform (eg, the Fcγ–Fel d 1 chimeric protein composed of the human Fcγ1 [γHinge-CHγ2-CHγ3], a flexible linker, and the major cat allergen [Fel d 1] or GFD) used the same mechanisms to alter the critical early signaling events responsible for the activation of mast cells/basophils (Fig 1), we investigated the role of GFD in IgE-dependent, FcεRI-mediated kinase phosphorylation. GFD is expected to indirectly coaggregate FcγRIIb and FcεRI through the formation of Fcγ–Fel d 1–IgE bridging (Fig 1, right panel). Tyrosine phosphorylation of Syk and ERK in Fel d 1–sensitized cells in response to allergen was markedly reduced in cells treated with GFD and likely is responsible for GFD's inhibition of basophil/mast cell function.[4] Thus as predicted, the antigen-specific platform uses the same negative signaling pathway to acutely block allergic reactivity.

A human bifunctional Fcγ-Fcε therapeutic protein

The first platform is a genetically engineered and expressed bifunctional human fusion protein that we have called GE2 and is comprised of the Fc portions of human IgE (CHε2–CHε3–CHε4) and IgG1 (hinge–CHγ2–CHγ3) joined by a flexible linker, a glycine-rich 15-amino-acid peptide that functions similar to the immunoglobulin hinge region to provide conformational bending for receptor binding. This platform was specifically designed to directly coaggregate the high-affinity FcεRI on basophils and mast cells with the inhibitory FcγRIIb receptor on these cells. It was predicted that such receptor coaggregation would inhibit their function in an allergen-nonspecific fashion. This molecule directly binds to FcγRII and FcεRI while the linker facilitates chain pairing, minimizes refolding and aggregation problems, and provides for flexibility between the 2 Fc regions.[3] The lead molecule, hGE2, has been studied extensively both in human systems in vitro and in vitro and in vivo in animal systems, including humanized mice. More recently, a mouse version of this has been constructed and tested by colleagues at Biogen-Idec (San Diego, Calif). This intervention is designed as a long-term systemic (subcutaneous) therapy

to be given on an ongoing basis for the treatment of any IgE-mediated disease, including severe food allergy. It is not designed as a "cure" or allergy tolerance–inducing therapy.

In vitro studies with hGE2 show it has a broad range of activity against the components of allergic disease

Initially, purified human blood basophils were passively sensitized with 10 μg/mL of a chimeric human anti–2-5-indo-4-hydroxy-3-nitrophenactyl (NIP) IgE, incubated with hGE2, and then triggered to degranulate with NIP-BSA. There was an average of 84% inhibition of mediator release with 10 μg/mL hGE2. Later experiments showed that when naturally sensitized basophils obtained from patients with cat allergy were incubated with hGE2 and then challenged with purified cat antigen, Fel d 1, hGE2 again showed a dose response, with 78% inhibition of Fel d 1–specific release at 10 μg/mL hGE2.[16] Importantly, hGE2 itself did not cause degranulation when it was incubated with basophils sensitized in vivo to cat or NIP. hGE2 not only blocked release of preformed mediators (eg, histamine) but also inhibited the production of newly formed inflammatory mediators (eg, TNF-α). In addition to effects on FcεRI-bearing basophils, we showed that hGE2 blocked IgE-driven responses from human cultured bone marrow–derived mast cells (BMMCs). Recent studies with the mouse homologue of hGE2 (mGE2, mouse Fcγ2a-mouse Fcε) have shown that this molecule inhibits preformed and newly synthesized allergic mediator release from mouse BMMCs, as well as the synergistic release of proallergic cytokines through Toll-like receptor 4 plus FcεRI stimulation. Critically, these effects were not seen with BMMCs from FcγRIIb-deficient mice and thereby proved the participation of FcγRIIb in the observed effects.[16] Recent studies suggest that human skin-derived mast cells might express FcγRIIa.[17] We are currently examining what affects GE2 has on FcεRI-mediated skin mast cell activation.

Langerhans-like dendritic cells and other antigen-presenting cells also express the high-affinity IgE receptor FcεRI, although in a form that lacks the β-chain. Such cells might play an important role in allergic inflammation through production of IL-16. When Langerhans-like dendritic cells were passively sensitized with antigen-specific human IgE and then challenged with antigen, they produced IL-16. However, when FcεRI and FcγRII were coaggregated with hGE2, IL-16 production was markedly inhibited.[12]

The effects of hGE2 on mediator release were shown to be time dependent; longer incubation with GE2 led to an increase in its ability to block FcεRI-driven mediator release. This result is not unexpected given the very slow off rate of IgE that is bound to FcεRI; hGE2 is not expected to instantaneously achieve maximum coaggregation. This result was paralleled in later in vivo findings in monkeys, in which there was a time-dependent increase in the effect of hGE2 on skin reactivity. Taken together, these approaches show that in vitro hGE2 can inhibit FcεRI-mediated proallergic effects on FcεRI-bearing human basophils, cord blood–derived mast cells, and Langerhans cells.

B cells express the low-affinity IgE receptor (CD23, FcεRII) and both FcγRIIa and FcγRIIb. Because an antibody to CD23 has been shown to inhibit isotype switching to IgE by human B cells,[18] we tested whether hGE2 could mediate a similar effect through CD23-FcγRII coaggregation. hGE2 potently inhibited various steps involved in IL-4 plus CD40–driven class-

switch recombination and IgE production. Inhibition resulted from a combination of blocking of initiation of ε germline transcription plus a direct effect on the process of isotype switching itself. Inhibition of class-switch recombination was dependent on CD23 binding and the phosphorylation of ERK and was mediated through suppression of IL-4–induced signal transducer and activator of transcription 6 phosphorylation.[18] Notably, this modification of the human B-cell isotype switch appeared to be associated with FcγRIIa expression, similar to what was observed on human skin mast cells. This effect might provide a second therapeutic benefit from hGE2 through downregulation of the afferent phase of the allergic response, but this remains to be shown in vivo.

In vivo studies with hGE2 confirm and extend evidence for its efficacy against allergic disease

We tested hGE2 in genetically modified mice that express the human FcεRI α chain, the FcεRI subunit that binds IgE. We demonstrated that hGE2 blocks the in vivo effector phase of the IgE response in the skin of such animals. Thus when skin sites were sensitized with human IgE to NIP or cat or peanut, administration of hGE2 at these sites showed a dose-dependent ability to block the skin test reactivity (passive cutaneous anaphylaxis [PCA]) at these sites to a systemic allergen challenge. Injection of hGE2 itself did not induce release of mediators, which otherwise might have accounted for an apparent later loss of reactivity or desensitization of GE2-injected sites.

mGE2, when administered as a parenteral treatment, as opposed to the local administration of hGE2 in transgenic mice, also showed a dose-dependent ability to block PCA reactivity to mouse IgE anti-dinitrophenol (DNP) antibody[16] in wild-type mice. To test the ability of mGE2 to block systemic reactivity, normal mice were systemically sensitized to DNP by means of administration of mouse IgE anti-DNP, such that challenge with DNP-BSA induced systemic anaphylaxis. Mice treated with mGE2 for up to 12 days before sensitization and challenge were protected from systemic allergic reactions.[16]

We took advantage of the fact that rhesus monkeys spontaneously produce IgE and manifest skin test reactivity to dust mites[19] to test whether hGE2 could inhibit Dermatophagoides farinae (a dust mite) skin test reactivity in nonhuman primates. D farinae–reactive monkeys were given graded intradermal injections with hGE2 (62.5-250 ng) or purified human IgE myeloma protein, and 5 hours later, the sites were challenged with D farinae at a dose optimized for each animal. hGE2 protein demonstrated significant inhibition at 250 ng per monkey, with maximal inhibition observed in 4 of 5 animals at 125 ng.[14] These results clearly indicate that hGE2 protein was able to inhibit naturally occurring, preexisting dust mite allergen–induced allergic skin reactivity in nonhuman primates in a dose-dependent fashion.

More recently, hGE2 was administered systemically (10 mg/kg) to Ascaris suum–sensitized cynomolgus monkeys, and animals were later skin tested with A suum extract. Inhibition of skin test reactivity increased from day 1 to maximal inhibition at day 14, and a positive effect was still evident at day 21 after the last hGE2 administration. The delay in maximal effect is likely due to the slow off rate of endogenous IgE with increasing GE2 binding over time. These results

indicated that hGE2 blocks allergic reactivity when systemically administered to nonhuman primates.[16]

Most recently, hGE2 has been used to treat cynomolgus monkeys (Macaca fascicularis) that are highly allergic to both D farinae and Dermatophagoides pteronyssinus dust mites (M. Van Scott, unpublished data).[20, 21] A single subcutaneous treatment with 10 mg/kg hGE2 led to a complete loss of lung reactivity to dust mite inhalation challenge in all 4 monkeys so treated and challenged 4 days and 4 weeks later. The beneficial effect was lost at 8 weeks. Treatment with two 5 mg/kg doses 2 weeks apart prevented the increase in respiratory reactivity in animals given a booster inhalation challenge of dust mite antigen. However, repeated dosing of monkeys with hGE2 was associated with the development of monkey antibody responses to both the human Fcγ and Fcε portions of hGE2 that might be predicted given that there is only an approximately 70% homology at the protein level between human and rhesus monkey (Macaca mulatta) IgE. These xenoantibodies (cross-species antibodies) lead to in vivo mast cell mediator release and serious adverse effects in some monkeys.

GE2 is expected to function as a long-term treatment that should be effective in polysensitized patients, including those with severe food allergy. Given its long half-life on FcεRI-binding cells, GE2 would be given as a monthly subcutaneous injection. Issues of hGE2 immunogenicity in human subjects will clearly need to be addressed. Notably, murine GE2 fails to demonstrate immunogenicity in mice (Kehry and Mertsching, unpublished observations).

A human cat chimeric Fcγ–Fel d 1 therapeutic protein

Traditional allergen immunotherapy relies on the cautious and protracted injection of gradually escalating amounts of the extracted allergen protein or proteins, and even so, immunotherapy can give rise to local and systemic allergic reactions.[4, 22] Because we had shown that the human Fcγ-Fcε fusion protein hGE2 that directly cross-links FcεRI and FcγRIIb was able to inhibit degranulation, we reasoned that a human Fcγ-allergen fusion protein would achieve a similar inhibitory effect in an allergen-specific fashion while preserving the immunogenicity of the allergen.

Thus the second platform we developed consists of genetically engineered and expressed chimeric human Fcγ-allergen protein, also joined through a flexible linker. This platform is designed to provide a novel and safe form of allergen-specific immunotherapy. We initially developed a human Fcγ-cat chimeric fusion protein, termed γ–Fel d 1 (or GFD), and composed of the human Fcγ1 (hinge-CH2-CH3) and the cat allergen Fel d 1 as a prototype and proof of principle for this novel form of allergen-specific immunotherapy.[4, 22]

In vitro studies with GFD show it inhibits allergic reactivity in an allergen-specific fashion

When freshly purified human basophils from patients with cat allergy were cultured along with increasing doses of GFD and followed by challenge with an optimal dose of purified Fel d 1, GFD at a dose of as little as 100 ng/mL inhibited histamine release up to greater than 90%. Similar inhibition was observed with cord blood mononuclear cells sensitized with serum from a patient with cat allergy. These same experiments showed that GFD does not function as an

allergen because release of mediators was not observed when Fel d 1 IgE-sensitized basophils were incubated with various doses of GFD, a finding that is critical if this approach is to be successful. Allergen specificity was shown by the fact that GFD did not block release from basophils sensitized in vitro with human anti-NIP IgE. Thus when the allergen portion of the Fcγ chimeric protein bound to specific IgE sitting in FcεRI on mast cells and basophils, the Fcγ portion simultaneously bound to and coaggregated the inhibitory receptor FcγRIIb, resulting in inhibition of allergic reactivity (Fig 1, right panel).

In vivo studies with GFD show it blocks allergic reactivity while functioning as an immunogen and lacking functional allergenicity

We next used PCA in human FcεRIα-transgenic mice to determine whether human GFD could block allergen-driven degranulation in vivo. Inhibitory effects of GFD through co–cross-linking FcεRI and FcγRIIb can be tested in these animals because their mast cells express the FcεRIα chain (and bind human IgE) and mouse FcγRIIb, which will bind to human IgG.[23] PCA reactivity was assessed by using human IgE to Fel d 1. We showed that reactivity in vivo was IgE dependent because it was lost after heating the human serum to 56°C for 30 minutes. Local GFD inhibited the IgE-driven PCA reactivity in a patient with cat allergy in a dose-dependent fashion, with GFD at 100 ng per spot completely blocking reactivity. As specificity controls, GFD did not induce mast cell release at anti-cat–sensitized sites nor did it inhibit PCA reactivity to human anti-NIP IgE. These results demonstrate that GFD is able to specifically block Fel d 1–induced allergic reactivity in vivo in an antigen-specific fashion.[4]

The immunotherapeutic ability of GFD was tested in a BALB/c mouse model of systemic reactivity in mice actively sensitized to Fel d 1. In such mice the murine FcγRs will bind the human Fcγ part of GFD, whereas the Fel d 1 portion functions as an antigen/allergen.22 BALB/c mice sensitized with Fel d 1 demonstrated local (skin), respiratory, and systemic reactivity to Fel d 1. Skin test reactivity and systemic reactivity (decrease of body temperature) were completely blocked by the acute administration of GFD treatment, as was Fel d 1–induced airway hyperresponsiveness (AHR) and eosinophilic airway inflammation.[22]

When GFD was administered in a protocol to mimic rush immunotherapy (eg, high-dose GFD administration in a short period), it was also able to inhibit Fel d 1–dependent allergic response, temperature change, AHR, allergic lung inflammation, and skin test reactivity in highly sensitized animals, and these effects persisted far longer than those observed in animals given a single GFD dose. Furthermore, the mice showed no evidence of reactivity to GFD administration, as opposed to the reactions observed in animals given “rush” immunotherapy with Fel d 1 alone. These results showed that established allergic responses to Fel d 1 could be ameliorated by GFD treatment, and beneficial immunomodulatory effects occurred when GFD was administrated in a regimen similar to rush allergen immunotherapy. Fel d 1–specific IgG1, IgG2a, and IgE responses were analyzed in response to rush GFD treatment to determine whether such treatment modulated established Fel d 1 antibody responses. GFD-treated animals had significantly increased Fel d 1 IgG1 antibodies compared with levels seen in untreated and Fel d 1–treated animals. Murine IgG1 is analogous to human IgG4 antibodies in its regulation but at the same time functions as a major allergic antibody in mice. GFD did not alter IgE or IgG2a protective antibody levels in the mice.[22] The short-term benefit of GFD is thus due to

GFD, by its presence, blocking allergen-specific responses. The mechanism by which GFD is expected to provide for long-term benefit is through its induction of a more balanced TH1/TH2 immune response to Fel d 1 in a fashion analogous to normal immunotherapy. The expectation is that because of its ability to act as an immunogen but block allergic reactivity to itself, GFD will be able to be administered as a very safe form of subcutaneous rush immunotherapy, such that a full course of immunotherapy can be rapidly achieved and treatment discontinued thereafter.

Because GFD contains Fel d 1, it was essential to determine whether GFD itself would induce allergic reactivity in vivo. Several experimental approaches showed this not to be the case. In contrast to Fel d 1, an equimolar amount of GFD did not induce a significant temperature decrease in Fel d 1–sensitized animals, intratracheally administered GFD did not induce AHR, and intradermal injection of GFD did not induce mast cell degranulation in the skin of Fel d 1–sensitized BALB/c mice.22 Taken together, these data strongly indicate that GFD itself does not elicit acute allergic reactivity in Fel d 1–sensitized animals. Plans for testing GFD in human subjects will require US Food and Drug Administration approval of appropriately manufactured and preclinically tested material.

Overall, we have shown that the chimeric human Fcγ–Fel d 1 allergen molecule functions as an immunogen while failing to acutely elicit allergic reactivity. These data suggest that the Fcγ-allergen chimeric protein approach in which the allergen is linked to the negative signaling FcγR ligand holds great promise as both a proof of concept for a novel immunotherapeutic approach and as a specific intervention against cat allergy. Such negative signaling immunotherapy should be able to be administered safely in high doses and a much briefer timeframe than conventional immunotherapy, with the only limitation being the time and dose necessary to induce the desired beneficial long-term modulation of the individual's immune/allergic response. Such an approach is particularly attractive for severe food allergy, in which many of the specific allergens are known and yet therapeutic options are severely limited.

Future directions

We believe that the human GE2 protein is ready for “prime time.” In collaboration with investigators at Biogen-Idec, we have tested a variety of variants with potential for improved function, and the currently identified molecule appears optimal.15 It remains to be determined whether the immunogenicity or other potential reactions to hGE2 given to human subjects will limit the development of this approach. The experiments in monkeys with hGE2 demonstrate both the promise and the potential risk. The only way one will ultimately answer this question is to test hGE2 in human subjects, as we well knew when we began this work.3 In mice antibodies to mGE2 have not been observed, despite repeated administration (Kehry, personal observation). Fortunately, a path forward is provided by testing phase I study subjects for the appearance of anti-hGE2 antibodies and, should they appear, analyzing the effects of those antibodies in vitro and in vivo by means of skin testing with hGE2.

The studies with GFD have shown the way forward in the development of allergen-specific immunotherapy based on Fcγ-containing chimeric proteins. This approach is expected to find its greatest applicability in the treatment of a limited number of allergens, those in which there are a small number of allergenic determinants, such as cat, and in particular in severe food allergy, in

which the risk of conventional immunotherapy is simply too great. Because, in contrast to hGE2, Fcγ-allergen proteins would not be administered as a systemic therapeutic, concerns about systemic reactivity would be the same or hopefully less than with conventional immunotherapy. Future quantitative skin testing of GMP produced GFD or other Fcγ-allergen chimeric proteins in known allergic subjects will provide key information about the likelihood of the success of this approach.

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