Chimeric human fcgamma-allergen fusion proteins in the prevention of allergy

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Abstract:

Allergic responses are strongly associated with Th2-type immune responses, and modulation of the skewed Th2 response toward a more balanced response is the major goal of allergen immunotherapy (IT) in allergic disorders. To achieve this goal, several approaches have been tested. The authors previously showed that a human immunoglobulin (Ig) Fcγ—Fcε fusion protein (GE2) that directly cross-links FcεRI and FcγRIIb on human mast cells and basophils was able to inhibit degranulation, and they reasoned that human gamma—allergen fusion protein would achieve a similar inhibitory effect in an allergen-specific fashion while preserving the immunogenicity of the allergen component. Therefore, the authors constructed and developed a human—cat chimeric fusion protein composed of the human Fcγ1 and the cat allergen Fel d1 (*Felis domesticus*) for cat allergen—specific IT. This article summarizes the therapeutic features and potential of this novel fusion protein for allergic IT.

Keywords: chimeric fusion protein | allergen immunotherapy | cat allergy | human mast cells | Fcγ | Fcε

Article:

Allergic responses are strongly associated with Th2-type immune responses, and modulation of the skewed Th2 response toward a more balanced response is the major goal of allergen immunotherapy (IT) in allergic disorders. To achieve this goal, several approaches have been tested, including allergen protein-, peptide-, modified allergen protein–, and allergy gene–based IT [1], [2], [3]. Traditionally, allergen IT has relied on the frequent injection of gradually escalated amounts of extracted allergen proteins. However, even when given according to a cautious and protracted schedule, standard allergen IT causes local and systemic allergic reactions and may elicit rare but life-threatening reactions [4], [5]. Therefore, investigators have great interest in developing novel forms of allergen IT in terms of safety and efficacy. The authors have shown that a human immunoglobulin (Ig) Fcγ–Fcε fusion protein (GE2) that

directly cross-links the high-affinity IgE receptors (FcεRI) and low-affinity IgG receptors (FcγRIIb) on human mast cells and basophils was able to inhibit degranulation [6], they reasoned that human gamma–allergen fusion protein would achieve a similar inhibitory effect in an allergen-specific fashion while preserving the immunogenicity of the allergen component. Therefore, the authors constructed and developed a human–cat chimeric fusion protein composed of the human Fcγ1 and the cat allergen Fel d1 (*Felis domesticus*) for cat allergen–specific IT [7], [8]. This article summarizes the therapeutic features and potential of this novel fusion protein for allergic IT.

Role of FcγRIIb in inhibiting allergic response

Cross-linking of the high-affinity IgE receptor (FcεRI) activates tyrosine phosphorylation of immunoreceptor tyrosine–based activation motifs(ITAMs) in the β and γ subunits of FcεRI in the cytoplasmic tails and leads to cell activation and degranulation in basophils and mast cells [4], [5]. This process leads to the classic immediate hypersensitivity reaction. The activation signal is balanced by the inhibitory receptors on these cells [9]. Human mast cells and basophils express the low-affinity IgG receptor (FcγRIIb), which contains an immunoreceptor tyrosine–based inhibitory motif (ITIMs) within its cytoplasmic tail [9]. Coaggregation of FcεRI to FcγRIIb has been shown to block in vitro and in vivo human basophil and mast cell function [10], [11], [12], [13]. This inhibition is mediated through reduction in tyrosine phosphorylation of Syk, ERK, and several other cellular substrates, and increased tyrosine phosphorylation of the adapter protein downstream of kinase (Dok), growth factor receptor–bound protein 2 (Grb2), and SH2 domain containing inositol 5-phosphatase (SHIP) [8], [14]. A previously developed Fcγ–Fcε fusion protein, GE2 [6], exhibited its inhibitory role in allergic responses in an allergen-nonspecific manner through directly cross-linking the FcεRI and FcγRIIb on mast cells and basophils. A more detailed review of this subject was published elsewhere [15].

Fcγ–Fel d1 fusion protein inhibits Fel d1–mediated allergic degranulation

To explore whether allergic responses can be modified in an allergen-specific manner through indirect co–cross-linking of the FcγRIIb and FcεRI bound the allergen-specific IgE, the authors genetically linked an allergen molecule using a flexible linker to a human Fcγ region. Because Fel d1 is the dominant allergen for cat allergy, and cat allergy is a major clinical problem, the authors constructed a chimeric human–cat protein composed of the hinge-through-CH$_3$ portion of human IgGγ1 Fc region fused to Fel d1 [7].

To test the efficacy of a chimeric human–cat fusion protein (GFD) on degranulation, freshly purified human basophils were purified from patients who had cat allergy, and were cultured along with various doses of GFD ranging from 1 ng/mL to 1 μg/mL, followed by the challenge with an optimal dose of purified Fel d1. GFD at 10 ng/mL inhibited histamine release by more than 75% (*P*<.002), whereas at 100 ng/mL, inhibition reached more than 90% (*P*<.001) (Fig. 1A). Similar inhibition was observed in cord blood–derived mast cells sensitized with cat-allergic serum. At 10 μg/mL, GFD reduced FcεRI-mediated release by an average of 77% (*P*<.05) in a dose-dependent fashion (Fig. 1B). Critically, these results also showed that GFD does not function as an allergen because prerelease of mediators was not observed when Fel d1–

sensitized basophils were incubated with GFD. These results indicated that GFD was able to block the Fel d1-induced degranulation of human mast cells and basophils, and that fusion of Fel d1 to the Fcγ altered the allergen nature of the Fel d1.
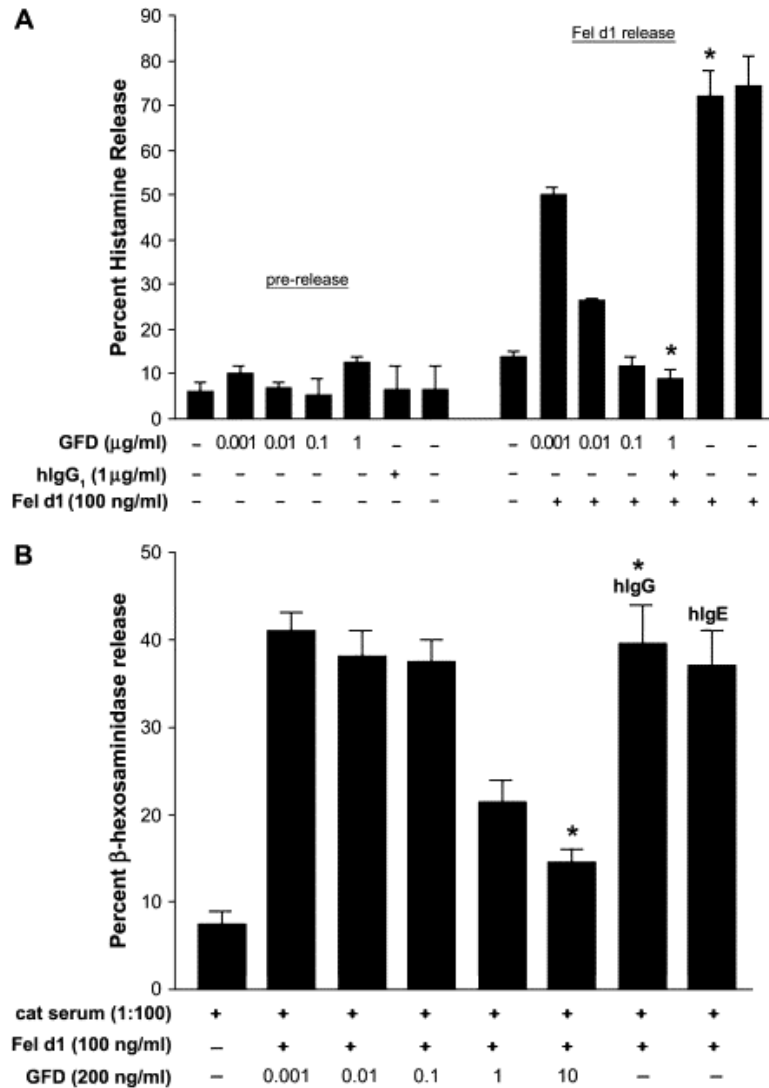

Fig. 1. GFD inhibits fresh human basophil and mast cell degranulation. (*A*) Dose-dependent inhibition of basophil histamine release by GFD. Basophils from an atopic donor were incubated for 2 hours with GFD and the supernatant assayed for histamine (pre-release). Cells were washed and then challenged with Fel d1 and histamine was measured in the supernatant (Fel d1 release). (*B*) Dose-dependent inhibition of fresh cord blood-derived mast cell β-hexosaminidase release using GFD. The results from one experiment, performed in duplicate, are representative of three separate donors. The asterisk indicates a statistically significant difference when comparing the two conditions. Human IgG and IgE are represented as hIgG and hIgE, respectively. (*Adapted from* Zhu D, Kepley CL, Zhang K, et al. A chimeric human–cat fusion protein blocks cat-induced allergy. Nat Med 2005;11:446–9; with permission.)

Fcγ–Fel d1 fusion protein inhibits signal events associated with degranulation

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 (MAP) kinases ERK1/2 and Syk are quickly phosphorylated in IgE-stimulated

human FcεRI–positive cells [16], [17]. To determine whether GFD is able to alter these critical early signaling events responsible for the early activation of mast cells and basophils, the authors investigated the role of GFD in IgE-dependent, FcεRI-mediated kinase phosphorylations. Cross-linking FcεRI on cord blood mast cells with IgE directed to Fel d1 induces substantial tyrosine phosphorylation of Syk and ERK, which was markedly reduced in cells preincubated with GFD (Fig. 2). Inhibition was observed 2 minutes after antigen stimulation and persisted as long as 15 minutes. Therefore, GFD coaggregation of FcεRI and FcγRII through formation of a Fcγ–Fel d1–IgE complex inhibits IgE-mediated Syk and ERK phosphorylation and is probably responsible for inhibiting basophil and mast cell function.
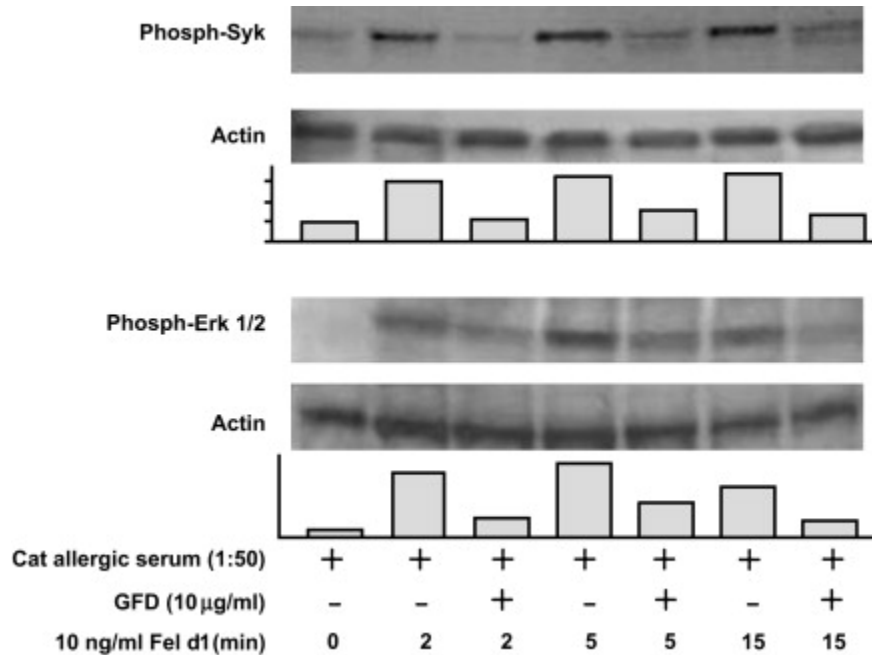

Fig. 2. GFD inhibits FcεRI-mediated Syk and ERK phosphorylation. Cord blood–derived mast cells were sensitized with cat-allergic serum, washed, and Western blotted with the indicated antibodies. Results are representative of three separate experiments. (*Adapted from* Zhu D, Kepley CL, Zhang K, et al. A chimeric human–cat fusion protein blocks cat-induced allergy. Nat Med 2005;11:446–9, with permission.)

Fcγ–Fel d1 fusion protein blocks passive cutaneous anaphylaxis reaction in FcεRIα transgenic mice

Allergic degranulation of mast cells in vivo can be determined with a passive cutaneous anaphylaxis (PCA) assay in transgenic (Tg) mice expressing human FcεRIα [6], [7]. After the back skin of the FcεRIα Tg mice is passively sensitized with human IgE from patients who have cat allergy and the mice undergo subsequent challenge with the appropriate antigen, results of the PCA are positive. Because the mast cells in these Tg mice also express the murine FcγRIIb that binds to human IgG [6], the inhibitory effects of GFD through co-crosslinking the humanized FcεRI and murine FcγRIIb can be tested using this PCA model.

As shown in Fig. 3A, GFD inhibited the IgE-mediated PCA of a patient allergic to cats in a dose-dependent manner (panel I–IV), and GFD at 100 ng per spot completely blocked the PCA (panel IV). Analogous inhibition was also observed by using GE2 fusion protein (Fig. 3B). However,

GFD exhibited higher efficacy for blocking PCA reactivity compared with GE2 (see Fig. 3B), with at least tenfold less amounts of GFD required for complete blocking of PCA (see Fig. 3B, panels III versus IV). GFD blocked PCA reactivity equally well when injected 4 hours after or simultaneously with the serum of a patient who had cat allergy (see Fig. 3B, panel III versus II). The purified IgE-dependent PCA of the patient who had a cat allergy, as shown by the inactivation of the PCA activity by heating the IgE with 56°C for 30 minutes [18], was also completely blocked by GFD. As a specificity control, GFD did not inhibit PCA reactivity to the human anti-NP (4-hydroxy-3-nitrophenylacetyl) IgE (data not shown). GFD itself was not inducing mast cell release at sensitized sites (data not shown). These data showed that GFD is able to block the Fel d1 allergen–specific allergic response in vivo.
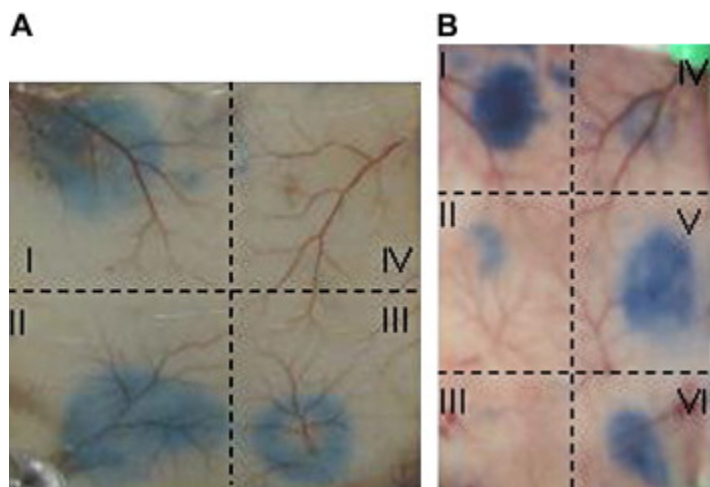

Fig. 3. In vivo GFD inhibits IgE-mediated degranulation in transgenic mice expressing human FcεRIα. (*A*) Dose-dependent inhibition of PCA by GFD. The labeled back skin sites were sensitized with cat-allergic serum (1:5 dilution) for 4 hours, followed by the administration of (I) saline; (II) 1 ng of GFD in 50 μL saline; (III) 10 ng of GFD; and (IV) 100 ng of GFD. (*B*) Comparison of GFD and GE2 for their ability to inhibit PCA reactivity to human anti–Fel d1 IgE. The skin sites were sensitized with 1:5 diluted cat-allergic serum, with the following treatment: (I) saline injection 4 hours later; (II) 100 ng GFD 4 hours later; (II) 100 ng GFD simultaneously with serum; (IV) 1 μg GE2 simultaneously with serum; (V) 100 ng GE2 4 hours later; and (VI) 100 ng GE2 simultaneously with serum. (*Adapted from* Zhu D, Kepley CL, Zhang K, et al. A chimeric human–cat fusion protein blocks cat-induced allergy. Nat Med 2005;11:446–9; with permission.)

Fcγ–Fel d1 fusion protein blocks the allergic responses in a mouse model

A Balb/c mouse model of systemic reactivity to Fel d1 in actively sensitized mice was used to test the immunotherapeutic ability of GFD. The rationale for this murine model to test the effects of human IgG Fc–Fel d1 fusion protein GFD is based on the fact that the murine Fc receptors for IgG (FcγRs) will bind human IgG Fc [13]. Thus, the Fc portion of GFD is expected to bind murine FcγRs, including FcγRIIb, which contains the ITIM that drives inhibitory signaling. The Fel d1 portion of GFD will also bind to murine Fel d1–specific IgE or IgG1 on the surface of sensitized mast cells and basophils.

BALB/c mice were sensitized with Fed d1 and treated according to the protocol diagrammed in Fig. 4A. The core body temperature, which was used as the indicator for systemic anaphylaxis, dropped by an average of 1.7°C ± 0.2°C starting at 5 minutes after the challenge (Fig. 4D). This decreased body temperature was completely blocked by the GFD treatment

($P<.001$). GFD treatment also completely blocked Fel d1–induced airway hyperresponsiveness (AHR) (Fig. 4B), as assessed through pulmonary resistance after methacholine challenge. Similarly, the eosinophilic airway inflammation in Fel d1–sensitized and intratracheally challenged mice, which was evident through increased eosinophils in bronchoalveolar lavage fluid, was blunted by GFD treatment (Fig. 4C). These results indicate that allergic responses to Fel d1 could be ameliorated by GFD treatment and these beneficial immunomodulatory effects occurred when GFD was administrated in a regimen similar to allergen IT after initial allergen sensitization. Anti–Fel d1 IgG1, IgG2a, and IgE responses induced by Fel d1 immunization showed variable changes in response to GFD treatment, but these changes did not reach statistical significance (data not shown). These results indicated that a protocol of allergen IT significantly inhibited allergic response to Fel d1 in a mouse model.
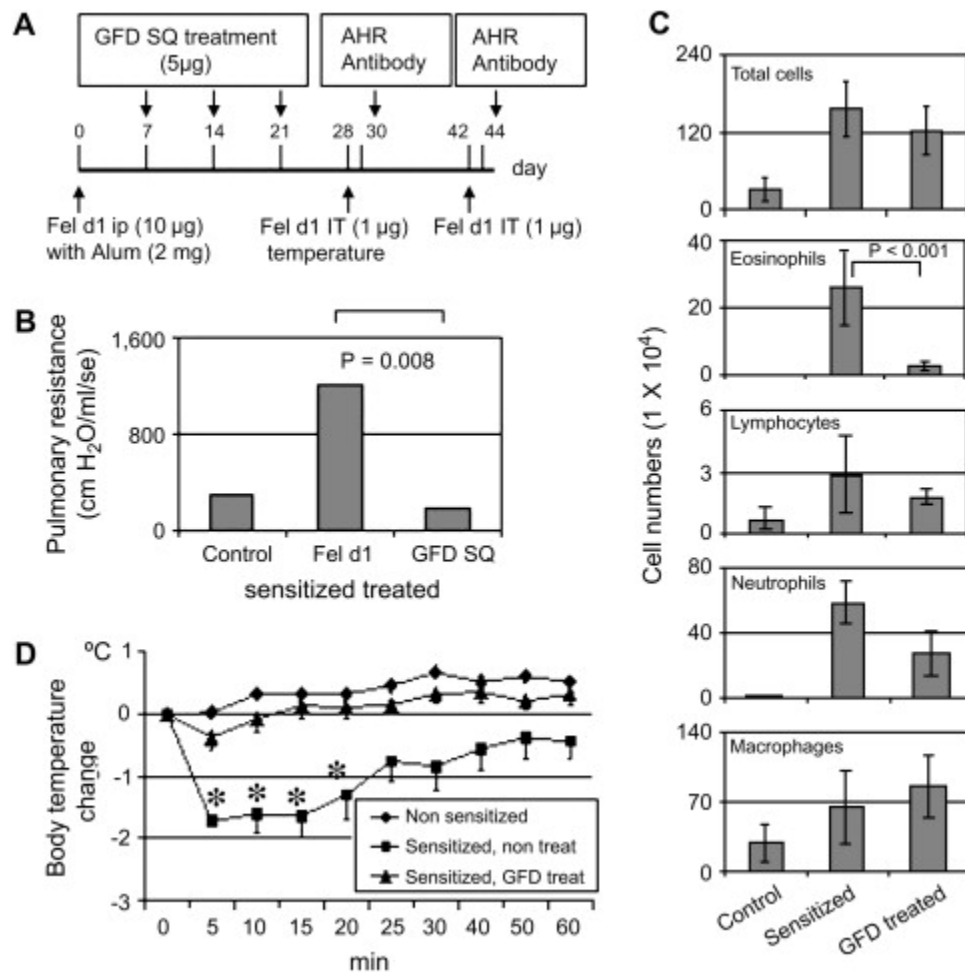

Fig. 4. Subcutaneous (SQ) administration of GFD blocked Fel d1–induced allergic response in a mouse model. (*A*) Schematic diagram of the experimental protocol. (*B*) Effect of GFD on blocking Fel d1–induced systemic allergic reactivity. The body temperature changes were assessed immediately after Fel d1 IT challenge (1 μg) with 5-minute intervals. The asterisk indicates a statistically significant difference between the two conditions. (*C*) Effect of GFD on Fel d1–induced AHR. The airway resistance to methacholine challenge was assessed using a computer-controlled small-animal FlexiVent® ventilator 2 days after IT Fel d1 challenge. The numbers represent the average values from three measurements of the airway resistance from a group of mice for each condition. (*D*) Effect of GFD on Fel d1–induced pulmonary eosinophilic inflammation. Total and differential numbers of bronchoalveolar lavage fluid cells were counted. (*Adapted from* Zhu D, Kepley CL, Zhang K, et al. A chimeric human–cat fusion protein blocks cat-induced allergy. Nat Med 2005;11:446–9; with permission.)

Fcγ–Fel d1 fusion protein fails to induce local or systemic reactivity on administration to Fel d1–sensitized animals

Because GFD is a fusion protein containing Fel d1, whether GFD itself would function as Fel d1 to induce allergic reactivity was important to determine. The authors undertook several approaches to examine this issue. As shown in Fig. 1A, GFD alone was not able to mediate histamine release from human basophils of patients who had cat allergy. In Fel d1–sensitized mice, Fel d1 induced a systemic allergic reactivity as shown through significant body temperature reduction (2.58°C ± 0.4°C). In contrast, an equimolar amount of GFD did not induce a significant temperature reduction in sensitized animals. In addition, IT administration of GFD did not induce AHR, as was seen with Fel d1. Intradermal injection of Fel d1 induced mast cell degranulation in the skin of Fel d1–sensitized BALB/c mice, but GFD did not induce the skin reactivity in sensitized animals. These data strongly indicated that GFD failed to elicit allergic reactivity systemically in the skin or airways of Fel d1–sensitized animals.

Fcγ–Fel d1 fusion protein blocks Fel d1–induced allergic reactivity in rush immunotherapy settings

Using the experimental regimen capable of blocking Fel d1–mediated allergic responses in a BALB/c mice model, the authors further sought to test whether GFD, when administered in a protocol to mimic rush IT (eg, high–dose GFD administrated in a short period), was able to inhibit Fel d1–dependent allergic responses in already highly sensitized animals, and whether a single administration of GFD was sufficient to acutely block reactivity in animals with established Fel d1–induced allergic responses.

The Fel d1–induced systemic reaction, measured using core temperature changes, was inhibited for a longer duration in the rush IT setting in which GFD was administered three times, whereas only acute, but not delayed, systemic reaction was inhibited by the single administration of GFD [8], in a similar manner as shown in Fig. 4D. Administration of GFD as the rush IT in the sensitized mice also blocked skin mast cell degranulation, assayed using an active skin test [8].

Fel d1–induced airway responsiveness and allergic lung inflammation were blocked by the rush IT protocols. Treatment with GFD completely reversed the Fel d1–induced airway hyperresponsiveness to methacholineand blunted the airway allergic inflammation, as shown through the significantly decrease either in the percentage or absolute numbers of eosinophils in the bronchoalveolar lavage fluid (*P*<.001). Histologic examination showed that Fel d1–induced pulmonary inflammation (Fig. 5A) was significantly inhibited by GFD administration (*P*<.001) (Fig. 5B). Marked goblet cell metaplasia was observed in the large airways of sensitized mice and Fel d1–treated mice, whereas GFD treatment inhibited this goblet cell metaplasia (Fig. 5C). These results indicted that Fel d1–induced allergic responses were significantly blocked by the rush IT protocol with higher-dose GFD administration.
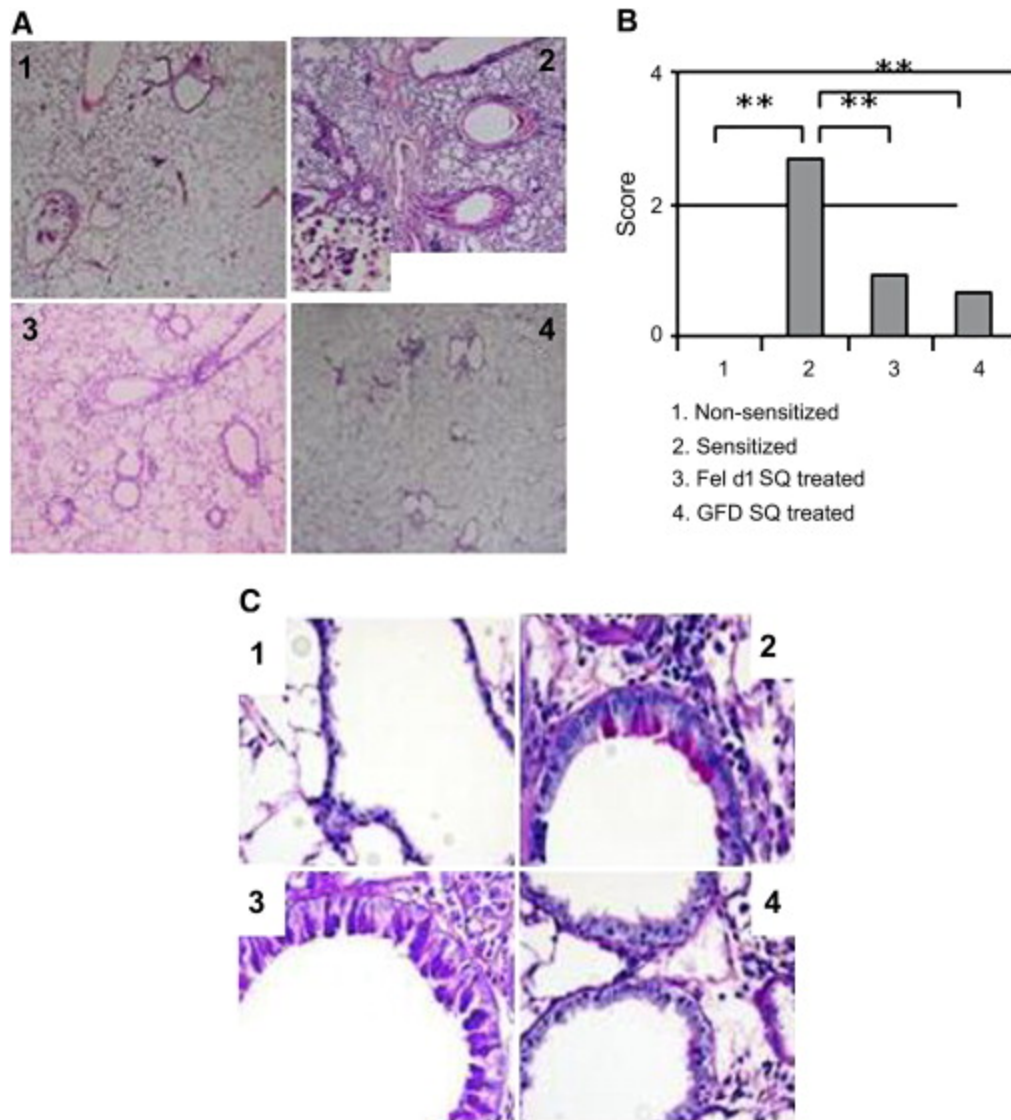
Fig. 5. Lung histologic changes in GFD–treated mice. (*A*) Light photomicrographs of hematoxylin and eosin–stained sections of lung tissue from the different treatments. Few leukocytes were observed in the lung of nonsensitized mice (*panel 1*). In contrast, numerous eosinophils were present after Fel d1 challenge in the control mice (*panel 2*). Both Fel d1 and GFD treatments (*panels 3 and 4*) led to markedly decreased eosinophil accumulation in the lung. (*B*) Semiquantitative analysis of the eosinophil accumulation in the lungs of animals challenged with IT with Fel d1 at day 54. Results of each group are expressed as mean ± SD. The asterisks indicate $P < .001$ between the groups. (*C*) Light photomicrographs of periodic acid-Schiff–stained sections of lung tissue from nonsensitized mice (*panel 1*) and mice receiving the different treatments. Marked goblet cell metaplasia was observed in large airways in the sensitized–challenged animals (*panel 2*). GFD treatment (*panel 4*), but not Fel d1 (*panel 3*), clearly inhibited this goblet cell metaplasia (*Adapted from* Terada T, Zhang K, Belperio J, et al. A chimeric human-cat Fcgamma-Fel d1 fusion protein inhibits systemic, pulmonary, and cutaneous allergic reactivity to intratracheal challenge in mice sensitized to Fel d1, the major cat allergen. Clin Immunol 2006;120:45–56; with permission.)

Fcγ–Fel d1 fusion protein immunotherapy modulates the antibody response to Fel d1

To examine whether the administration of GFD with rush IT protocol was able to modulate the antibody responses to Fel d1, the Fel d1–specific IgG1, IgG2a, and IgE were analyzed. The Fel d1 sensitization and challenge induced significant increases in serum Fel d1 antibodies, with

levels ranging from undetectable (<1.0 U/mL) for nonsensitized animals to geometric means of 57,859, 2, and 113 U/mL for IgG1, IgE, and IgG2, respectively ($P<.01$ for all), reflecting the Th2–dominant allergic antibody responses. GFD treatment led to increased IgG1 antibodies to Fel d1 compared with untreated (geometric means of 186,167 versus 57,859 U/mL; $P<.05$) and Fel d1–treated animals (186,167 versus 33,157 U/mL; $P<.02$). GFD did not alter IgE or IgG2 antibodies (geometric means of 34.4 and 166 U/mL, respectively) and Fel d1 treatment did not significantly alter any antibodies levels (geometric means of 33,157, 14.2, and 140 U/mL for IgG1, IgE, and IgG2a, respectively) compared with untreated animals.

Potential use of Fcγ–Fel d1 fusion protein for allergic blockade and immunotherapy in cat allergy

Data show that the chimeric GFD protein is a promising model for a new form of IT in allergy and a specific intervention against cat allergy. The advantages of this approach are that the allergen carries its own negative signal, the Fcγ portion that has been shown to drive inhibitory signaling in human mast cells and basophils. GFD's indirect cross-linking of FcγRIIb and FcεRI through naturally occurring IgE to Fel d1 results in an acute antigen–specific inhibition of mediator release. As a result, from a safety perspective, GFD should be able to be given 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 allergic response to cats. If successful, a similar approach could be undertaken in severe food allergy, where many of the specific allergens are known and therapeutic options are severely limited.

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