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A chimeric human-cat Fcγ-Fel d1 fusion protein inhibits systemic, pulmonary, and cutaneous allergic reactivity to intratracheal challenge in mice sensitized to Fel d1, the major cat allergen

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Abstract Co-aggregation of FcRI with FcγRIIb can block FcRI-mediated reactivity and Fc gamma:allergen chimeric proteins, by co-crosslinking FcγRIIb to allergen-specific IgE bound to the FcRI can block allergen-specific reactivity. We evaluated whether a human cat chimeric fusion protein (GFD) composed of part of the human Ig G1 Fc fused to the major cat allergen (Fel d1) would function as allergen immunotherapy while not inducing acute allergic reactivity in mice sensitized to Fel d1. Injection of GFD 6 h prior to Fel d1 challenge acutely blocked systemic and skin reactivity to Fel d1 challenge while mice given subcutaneous immunotherapy with GFD at days 37, 38, and 39 showed inhibition of systemic, lung, and cutaneous reactivity to Fel d1 2 weeks later. GFD immunotherapy did not induce systemic reactivity. Overall, the Fcγ-Fel d1 chimeric fusion protein blocked Fel d1-induced IgE-mediated reactivity but did not induce in vivo mediator release on its own; suggesting that this approach using allergen combined with Fc gamma1 so as to achieve inhibitory signaling may provide an enhanced form of allergen immunotherapy.
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Introduction

For nearly 100 years, parenteral administration of allergens as immunotherapy has been used as a disease modifying therapy in allergic respiratory diseases [1,2]. However, even when given in a cautious and protracted schedule, standard allergen immunotherapy gives rise to local and systemic allergic reactions and carries the risk of eliciting rare but life-threatening reactions [3,4]. Furthermore, immunotherapy with food allergens has proven to be both dangerous and of limited efficacy [5,6]. Thus, there is great interest in the development of novel forms of allergen immunotherapy.

Allergen exposure, be it from natural sources or as a result of immunotherapy, results in cross-linking of FcRI, which activates tyrosine phosphorylation of immunoreceptor tyrosine-based activation motifs (ITAMs) in the β- and γ-FcRI subunit cytoplasmic tails and leads to cell activation and degranulation in basophils and mast cells [7]. This, in turn, leads to the classic immediate hypersensitivity reaction. Human mast cells and basophils also express FcγRIIb, which contains an immunoreceptor tyrosine-based inhibitory motif (ITIMs) within its cytoplasmic tail [8,9]. Co-aggregation of FcRI to FcγRIIb has been shown to block in vitro and in vivo human basophil and mast cell function [10—13]. Our previously reported studies experiments have shown that this inhibition is mediated via 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 (Dok) growth factor receptor-bound protein 2 (Grb2) and SH2 domain containing inositol 5-phosphatase (SHIP) [8,14].

We set out to test in vivo whether, by driving co-aggregation of FcγRIIb and FcRI, an allergen molecule combined with a human Fcγ region would: (1) block allergen driven reactivity, (2) fail to function as an allergen, and (3) serve as an immunogen for allergy immunotherapy. We employed a novel chimeric human: cat protein composed of part of the human IgGγ1 Fc region fused to Fel d1, the major allergen produced by domestic cats. Previous experiments had shown that Fcγ-Fel d1 chimeric fusion protein (GFD) inhibited allergen-driven IgE-mediated mediator release in vitro from human basophils and cord blood-derived mast cells, and in vivo in passive cutaneous anaphylaxis in FcRIα transgenic mice sensitized with human IgE to Fel d1 [14].

In the current study, we tested whether mice actively sensitized to Fel d1, when treated with the gamma-Fel d1 fusion protein, were protected from local, airway, and systemic reactivity. A single systemic administration of GFD could block type I allergic reactivity acutely, while subcutaneous immunotherapy with GFD at days 37, 38, and 39 showed inhibition of systemic, lung, and skin test reactivity, at the same time. GFD itself did not trigger allergic reactivity in Fel d1-sensitized mice even when given in up to 5-fold higher levels than Fel d1.

Materials and methods

Animals

Male Balb/c mice were purchased from Harlan Sprague—Dawley Inc. (Indianapolis IN) and housed in the UCLA Vivarium under specific pathogen-free conditions. The Chancellor's Animal Research Committee approved all of the animal studies as adhering to the guidelines set forth by the National Institutes of Health. Mice were 6—8 weeks of age at the initiation of each experiment.

Gamma Fel d1 (GFD) fusion protein

The Fcγ-Fel d1 chimeric fusion protein was produced by expression of a chimeric gene consisting of the genomic human IgGγ1 constant region from the hinge through CH3, a 15 amino acid linker, and a combined Fel d1 chain 1 and 2 construct kindly provided by Drs. Amanda Sun and Paul Guyre at Dartmouth Medical School as described [14]. SDS-PAGE demonstrated that the Fcγ-Fel d1 fusion protein was expressed as the predicted ~140 dimer. Western-blot analysis and ELISA demonstrated that both antibodies specific for the human γ chain and Fel d1 antigen recognized the GFD protein.

Fel d1 sensitization and GFD treatment protocols

Protocol 1
On days 0 and 14, mice were sensitized by means of an IP injection of 5 μg of purified natural Fel d1 (Indoor Biotechnology Inc., Charlottesville, VA) emulsified in 2 mg of alum hydroxide (Alhydrogel; Brenntag Biosector, Denmark) in a total volume of 160 μl. On days 28, 29, 30, and 33, the sensitized mice were boosted with an intratracheal (IT) administration of 1 μg of native Fel d1. As immunotherapy in this protocol, native Fel d1 (5, 10, and 20 μg/100 μl) or GFD (10, 20, 40 μg/100 μl) in normal saline was given SQ on days 37, 38, and 39, while control treatment consisted of the same volume of saline alone (Fig. 1A). Twice the dose of GFD was used for Fel d1, as the molecular weight of GFD is approximately twice that of Fel d1. Animals were given an IT challenge with 1 μg of native Fel d1 on days 40, 47, and 54. Systemic reactivity (temperature) was examined at day 54, airway hyper-responsiveness (AHR), airway, and lung allergic inflammation (e.g., eosinophilia) were tested on day 56, and skin sensitization was examined on day 60.

Protocol 2
Mice were sensitized by IP injection of either 1 or 10 μg of purified natural Fel d1 emulsified in 2 mg of alum hydroxide in a total volume of 160 μl at day 0 (Fig. 1B), boosted IT with 1 μg of Fel d1 on day 28, and challenged IT with 1 μg of Fel d1 on days 35 and 49. As an acute treatment regimen, 5 μg of GFD in 50 μl of 0.9% NaCl was given by IP injection 6 h before the IT challenge with Fel d1 on day 35. For sham treatment, the same volume of saline was given IP.

Measurement of airway response to methacholine

Airway responsiveness was measured 48 h after IT challenge using a modified forced oscillation method as previously described [15—19]. Briefly, mice were anesthetized with 90 mg/kg of pentobarbital sodium injected IP and anesthesia was confirmed by the absence of response to paw pinch. The mice were then tracheostomized with a 22-gauge IV catheter (Terumomedical Corp., Elkton, MD) that was firmly tied in place. Mice were then connected to a computer-

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