Targeting Fel d 1 to FcγRI induces a novel variation of the T_H2 response in subjects with cat allergy

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Background: Induction of $CD4^+$ T cells that produce IL-10 or IFN-γ is central to the protective effects of conventional allergen immunotherapy.
Objective: We examined the T-cell modulatory capacity of a fusion protein (H22–Fel d 1) that targets Fel d 1 to the high-affinity IgG receptor (FcγRI) on antigen-presenting cells.
Methods: Monocyte-derived dendritic cells pulsed with H22–Fel d 1 were analyzed for surface phenotype and cytokine secretion by flow cytometry and cytometric bead assay, respectively. $CD4^+$ T cells generated after coculture with H22–Fel d 1—pulsed dendritic cells were analyzed at the single-cell level by flow cytometry after intracellular cytokine staining. The T-cell repertoire was compared for subjects with (IgE^+) and without cat allergy ($IgE^{neg}IgG^{neg}$), including subjects with a modified T_H2 response ($IgE^{neg}IgG^+$).
Results: H22–Fel d 1 induced a semimature phenotype in dendritic cells in conjunction with a selective increase in $IL\text{-}5^+$ and $IL\text{-}10^+$ $CD4^+$ T cells compared with nonreceptor-targeted Fel d 1. Amplified T cells included diverse subtypes characteristic of T_H0 ($IL\text{-}5^+IFN\text{-}\gamma^+$), regulatory T_H1 ($IL\text{-}10^+IFN\text{-}\gamma^+$) and regulatory T_H2 ($IL\text{-}10^+IL\text{-}5^+$) cells. T-cell qualitative changes were restricted to subjects with allergy and were distinct from a modified T_H2 response. Blocking IL-10 induced by H22–Fel d 1 selectively increased $IL\text{-}5^+$ $CD4^+$ T cells, suggesting that T_H2 responses were controlled.
Conclusion: Targeting Fel d 1 to FcγRI induces a novel variation of the T_H2 response that incorporates major elements of a protective T-cell response.
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Key words: *CD64, IL-10, IL-5, IFN-γ, H22–Fel d 1, dendritic cells, $CD4^+$ T cells, regulatory T cells, immunotherapy*

Abbreviations used
APC: Antigen-presenting cell
MCP: Monocyte chemoattractant protein
MDDC: Monocyte-derived dendritic cell
MIP: Macrophage inflammatory protein
TARC: Thymus and activation-regulated chemokine

Conventional immunotherapy, which involves repeated injection of allergen extracts, remains the mainstay of treatment for allergy to a variety of inhalant allergens. Although there is considerable evidence for its efficacy, this approach has several drawbacks, including a risk for anaphylaxis and prolonged treatment regimens. Although the immune mechanisms which underlie the protective effects of immunotherapy remain ill-defined, there is evidence that induction of $CD4^+$ T cells, which secrete IL-10 or IFN-γ, is a prerequisite. This suggests the involvement of T cells that are either regulatory or T_H1-like.[1-4] Consequently, recombinant allergens or allergen variants, which may provide a more tailored approach to immunotherapy, have been engineered with a view to activating specific T-cell subsets preferentially.[5-7] Unfortunately, many allergens induce weak T-cell responses *in vitro*, which may reflect the low precursor frequency of allergen-specific T cells in the T-cell repertoire. As a result, defining T-cell mechanisms that underlie the protective effects of immunotherapy has proved to be a challenge. Moreover, *in vivo* presentation of allergen to T cells during conventional immunotherapy is likely to be inefficient.

The approach described here uses a fusion protein designed to target allergen to the surface of antigen-presenting cells (APCs) as a method of improving allergen presentation to T cells. This protein (designated H22–Fel d 1) is composed of the major cat allergen, Fel d 1, linked to a single chain fragment of the variable region (sFv) of the humanized anti-CD64 mAb, H22. This molecule has been shown to bind to the high-affinity IgG receptor, FcγRI (CD64), on APCs.[8] Targeting to CD64 has been shown to increase uptake of diverse antigens by APCs and to increase T-cell proliferative responses.[8-11]

A unique feature of the major cat allergen, Fel d 1, is its ability to induce a modified T_H2 response (IgG^+IgE^{neg}) in a subset of individuals with high-level environmental exposure.[12] This protective response is associated with increased IL-10 production in PBMC cultures stimulated with major T-cell epitopes of Fel d 1.[4] Because targeting Fel d 1 to CD64 on APCs may enhance presentation of major T-cell epitopes, we hypothesized that H22–Fel d 1 would promote induction of IL-10–producing $CD4^+$ T cells. The current study was designed to examine the immunomodulatory effects of H22–Fel d 1 at both the APC and the T-cell level. To this end, we analyzed patterns of cytokine secretion by dendritic cells and performed rigorous single-cell analyses of cytokine

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TABLE I. Serum antibody profiles in subjects with distinct immune responses to cat allergen*

Group	Total IgE† (IU/mL)	IgE to cat† (IU/mL)	Fel d 1 IgE‡ (IU/mL)	Fel d 1 IgG§ (U/mL)
Allergic (n = 11)	119 (60-235)	13 (7-25)	7 (0.5-99)	3168 (1392-7213)
Modified T_H2 (n = 9)	22 (10-46)	<0.35	<0.35	4917 (1923-12573)
Control (n = 5)	14 (4-45)	<0.35	<0.35	<125

*Values represent geometric means (95% CIs).
†Measured by CAP assay.
‡Measured by streptavidin CAP assay.
§Measured by radioimmunoprecipitation assay.

production by $CD4^+$ T cells. Our findings provide evidence of a new approach to immunotherapy and present a new paradigm for variations of a T_H2 response.

METHODS

Classification of subjects

Patients were recruited from the University of Virginia Allergic Diseases Clinic or by advertisement. Subjects with cat allergy were classified on the basis of high-titer serum IgE antibodies to cat extract (CAP > 0.7 IU/mL) and anti—Fel d 1 IgE antibodies measured by streptavidin CAP assay.[13] Subjects with a modified T_H2 response to cat allergen were identified on the basis of high-titer anti–Fel d 1 IgG antibodies measured by antigen binding radioimmunoassay (>500 U/mL) without anti–Fel d 1 IgE antibodies.[12,14] Control subjects had no measurable serum IgG or IgE antibodies to Fel d 1. Serum antibody profiles are shown in Table I. All studies were approved by the University of Virginia Human Investigations Committee.

Recombinant allergens and LPS

Recombinant Fel d 1 and Fel d 1 targeted to the high-affinity IgG receptor, FcγRI (H22–Fel d 1),[8] were expressed in *Pichia pastoris* and purified by multistep chromatography. The purity was >90% as determined by SDS-PAGE. The endotoxin content of Fel d 1 and H22–Fel d 1 was comparable (145 EU/mL and 153 EU/mL, respectively). LoTox Der p 1 (Indoor Biotechnologies Inc, Charlottesville, Va) (endotoxin content < 0.05 EU/μg) was purified from mite culture by affinity chromatography (>90% purity by SDS-PAGE) and endotoxin removed by proprietary techniques. LPS was obtained from Sigma-Aldrich (St Louis, Mo).

Proliferation assays

For a description of proliferation assays, see this article's Methods section in the Online Repository at www.jacionline.org.

Induction of cytokines in monocyte-derived dendritic cells

Dendritic cells were generated from $CD14^+$ monocytes (see this article's Methods section in the Online Repository at www.jacionline.org). Monocyte-derived dendritic cells (MDDCs)[15] were stimulated for 24 to 48 hours in medium containing 10% autologous human serum with allergen (10 μg/mL) or LPS (1 μg/mL). Cytokines were measured by cytometric bead assay (human 27-plex kit; BioRad, Hercules, Calif).

$CD4^+$ T-cell/MDDC cocultures

$CD4^+$ T cells were purified by negative selection using magnetic-activated cell sorting (Miltenyi Biotec, Auburn, Calif), and purity was assessed by flow cytometry (>95%). MDDCs were pulsed with allergen (10 μg/mL) for 48 hours before coculture (2×10^5) with purified $CD4^+$ T cells (8×10^5) for 10 days in 24-well plates. Cultures were supplemented with recombinant human IL-2 (12 U/mL; BioSource International, Carlsbad, Calif) on day 5, and intracellular cytokines were measured on day 10. In some experiments, a blocking anti–IL-10 receptor mAb (clone 3F9; BioLegend, San Diego, Calif) or isotype control (clone RTK2758; BioLegend) was added (20 μg/mL) on day 0 of coculture.

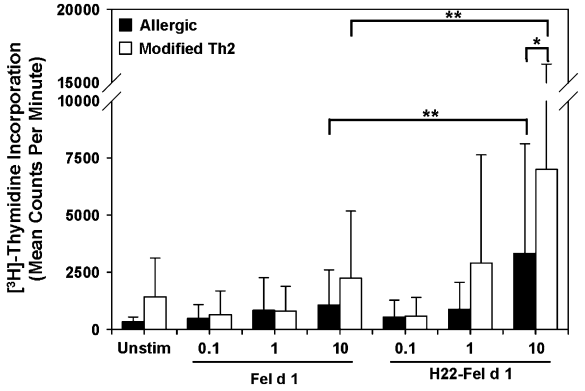

FIG 1. PBMC proliferation to H22–Fel d 1. PBMCs were stimulated with allergen and proliferation measured on day 7 (n = 7 per group). Values correspond to mean counts per minute + SDs. *$P < .01$; **$P < .001$. Subjects with cat allergy: $P < .04$ for unstim versus all other conditions. Modified T_H2 responders: $P < .02$ for unstim versus Fel d 1 (10 μg/mL) and H22–Fel d 1 (1 and 10 μg/mL). *Unstim,* Unstimulated.

Flow-cytometry analysis

For a complete list of fluorescently conjugated mAbs and instrumentation, see this article's Methods section in the Online Repository at www.jacionline.org.

Dendritic cell phenotyping

Monocyte-derived dendritic cells were collected immediately after generation or allergen pulsing (24-48 hours) and washed in cold FACS buffer (0.5% BSA, 2 mmol/L EDTA in PBS). Cells were stained for surface markers, washed, and analyzed by flow cytometry.

Intracellular cytokine staining

$CD4^+$ T-cell/MDDC cocultures were restimulated with phorbol 12-myristate 13-acetate (50 ng/mL) and ionomycin (2 μg/mL; Sigma-Aldrich) for 5 hours, and Brefeldin A (BD Biosciences, San Jose, Calif) was added during the final 4 hours. Cells were stained for surface and intracellular cytokines according to the manufacturer's instructions (Caltag International Inc, Carlsbad, Calif) and analyzed by flow cytometry.

Statistical analysis

The Student *t* test (SPSS 14.0, SPSS Inc, Chicago, Ill) was used to compare proliferation, cytokines, and mean fluorescence intensity levels. The