Chronic cat allergen exposure induces a T_H2 cell–dependent IgG_4 response related to low sensitization

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Background: In human subjects, allergen tolerance has been observed after high-dose allergen exposure or after completed allergen immunotherapy, which is related to the accumulation of anti-inflammatory IgG_4. However, the specific T-cell response that leads to IgG_4 induction during chronic allergen exposure remains poorly understood.

Objective: We sought to evaluate the relationship between cat allergen–specific T-cell frequency, cat allergen–specific IgE and IgG_4 titers, and clinical status in adults with cat allergy with and without cat ownership and the cellular mechanism by which IgG_4 is produced.

Methods: Fel d 1–, Fel d 4–, Fel d 7–, and Fel d 8–specific T-cell responses were characterized by CD154 expression after antigen stimulation.

Results: In allergic subjects without cat ownership, the frequency of cat allergen (Fel d 1 and Fel d 4)–specific T_H2 (sT_H2) cells correlates with higher IgE levels and is linked to asthma. Paradoxically, we observed that subjects with cat allergy and chronic cat exposure maintain a high frequency of sT_H2 cells, which correlates with higher IgG_4 levels and low sensitization. B cells from allergic, but not nonallergic subjects, are able to produce IgG_4 after cognate interactions with sT_H2 clones and Fel d 1 peptide or the Fel d 1 recombinant protein.

Conclusion: These experiments suggest that (1) allergen-experienced B cells with the capacity to produce IgG_4 are present in allergic subjects and (2) cat allergen exposure induces an IgG_4 response in a T_H2 cell–dependent manner. Thus IgG_4 accumulation could be mediated by chronic activation of the T_H2 response, which in turn drives desensitization. (J Allergy Clin Immunol 2015;136:1627-35.)

Key words: *Cat allergy, Fel d 1, Fel d 4, T_H2 cells, allergen tolerance, asthma, class II tetramer, CD154, IgG_4, allergen exposure*

An allergic reaction is characterized by a type 2 immune response in which $CD4^+$ T_H2 cells produce IL-4, IL-5, IL-13, IL-9, and IL-10; and allergen-specific IgE which binds to high-affinity IgE receptors (FcεRI) on basophils and mast cells to enhance histamine secretion and the allergic response.[1] Although the pathologic T_H2 response against allergen is well characterized, the nature of tolerant responses is less well understood. Naturally occurring allergen tolerance has been described in subjects with cat allergy, with a correlation between cat allergen exposure and reduced sensitivity observed in children owning a cat.[2]

Reduced sensitivity in subjects with cat ownership is associated with the accumulation of specific IgG_4, which has the capacity to attenuate IgE-mediated allergic symptoms.[3-6] IgG_4 accumulation is also observed in tolerant beekeepers during the bee sting season, confirming an important role for this antibody in allergen tolerance.[7] Allergen-specific T cells that produce IL-10 are observed in both cat owners and beekeepers and likely play a role in specific IgG_4 induction.[8,9] Furthermore, induction of IL-10 and specific IgG_4 is correlated with improved clinical outcome during allergen-specific immunotherapy.[10-12] Although the importance of these factors in the allergen tolerance mechanism is therefore evident, the role of T-cell subsets and other factors supporting specific IgG_4 production remains unclear.

Although IgE detection is routinely used to discriminate allergen sensitivity, characterization of allergen-specific $CD4^+$ T cells is more difficult. For subjects with cat allergy, the situation is further complicated by the presence of multiple allergens. Fel d 1 is recognized as the major cat allergen, and 80% to 95% of subjects with cat allergy have IgE that recognizes Fel d 1[13]; however, IgE to Fel d 4, Fel d 7, and Fed d 8 has been reported to be present in 60%, 40%, and 20% of subjects with cat allergy, respectively.[14,15] All of these allergens can be major $CD4^+$ T-cell targets in subjects with cat allergy.[16]

Peptide–MHC class II tetramer staining is one of the most sensitive methods to characterize antigen-specific $CD4^+$ T cells during inflammatory diseases and allergy.[17-19] Detection of CD154 expression after antigen stimulation has also been used to track allergen-specific $CD4^+$ T cells.[19-21] In the present study we used these T-cell tracking methods to characterize Fel d 1–, Fel d 4–, Fel d 7–, and Fel d 8–specific T-cell responses in adults

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Abbreviations used
APC: Allophycocyanin
CRTH2: Chemoattractant receptor–homologous molecule expressed on T_H2 cells
PE: Phycoerythrin
sT_H2: Specific memory $CD4^+$ T_H2
T_{FH}: T follicular helper

with cat allergy with and without cat ownership. The relationship between cat allergen–specific T-cell frequency, cat dander–specific IgE and specific IgG_4 titers, and clinical status was examined, and the cellular mechanism that led to high IgG_4 production in subjects with cat allergy was investigated. Here we demonstrate that chronic cat allergen exposure supports a T_H2 cell–dependent IgG_4 response.

METHODS

Subjects

Thirty-eight subjects were recruited from the Virginia Mason Medical Center Allergy Clinic and Benaroya Research Institute. All subjects were HLA typed (see the Methods section in this article's Online repository at www.jacionline.org). Subjects with cat allergy (n = 24) had a positive skin test response, serum IgE response, or both to cat dander of greater than 0.35 kU_A/mL, as determined by using the ImmunoCAP test (Phadia AB, Uppsala, Sweden), and had known allergic symptoms. Nonallergic subjects (n = 14) had negative IgE ImmunoCAP scores for cat allergens and other allergens (dust mite, Bermuda grass, timothy grass, alder, and *Alternaria* species). All subjects were recruited after obtaining informed consent and institutional review board approval. All assays were performed in a blinded manner with regard to cat ownership status and allergic scores. Asthma and rhinitis scores were based on symptoms and medications, as described in the Methods section in this article's Online repository.

Ex vivo analysis of cat allergen–specific $CD4^+$ T cells

For the CD154 expression assay, 30×10^6 PBMCs (at 6×10^6 cells/mL) were stimulated for 3 hours at 37°C with 5 μg/mL synthesized peptide (20 amino acids in length with a 12-amino-acid overlap) pools (Mimotopes, Notting Hill, Australia) spanning the entire Fel d 1 (p1-p18), Fel d 4 (p1-p20), Fel d 7 (p1-p18), and Fel d 8 (p1-p27) sequences or with dimethyl sulfoxide (control) in the presence of 1 μg/mL anti-CD40 (HB14; Miltenyi Biotec, Bergisch Gladbach, Germany) in 10% human serum RPMI medium. After stimulation, PBMCs were labeled with phycoerythrin (PE)–conjugated CD154 and $CD154^+$ cells and were enriched with anti-PE magnetic beads (Miltenyi Biotec). A one-tenth fraction of unenriched cells was saved for analysis for frequency determination (see the Methods section in this article's Online repository). After enrichment, cells were stained with appropriate antibodies (see the Methods section in this article's Online repository). Staining with HLA-DRB1*0301/Fel d 1 chain $2_{17\text{-}36}$ and HLA-DRB5*0101/Fel d 1 chain $1_{17\text{-}36}$ tetramers was carried out, as previously described.[18]

Intracellular cytokine staining

For *ex vivo* intracellular cytokine staining combined with the CD154 expression assay, monensin was added during stimulation (GolgiStop; BD Biosciences, San Jose, Calif), according to the manufacturer's instructions. *In vitro* intracellular cytokine staining combined with MHC class II tetramer staining was performed, as previously described.[19] Cells were stained with appropriate antibodies (see the Methods section in this article's Online repository).

Specific and total IgG_4 analysis

The determination of Fel d 1– and Fel d 4–specific IgG_4 antibody levels in adult serum was performed by using ELISA, as described previously.[22] Briefly, either 1 μg/mL recombinant Fel d 1 or Fel d 4 (prepared as previously described[15,16]) or 1% BSA (as a control) was coated on wells. After washing, one-tenth–diluted sera from patients were added to wells coated with Fel d 1 or Fel d 4 or 1% BSA. The detection methods are as described in the Methods section in this article's Online repository. Total IgG_4 levels were quantified in culture supernatants by using Human IgG4 Ready-SET-Go (eBioscience, San Diego, Calif), according to the manufacturer's instructions.

Fel d 1–specific T_H2 clone generation

After staining with DRB5*0101/Fel d 1 chain $1_{17\text{-}36}$ tetramers or DRB1*0301/Fel d 1 chain $2_{17\text{-}36}$, single memory ($CD45RA^-$) tetramer-positive cells were sorted *ex vivo* in a 96-well plate on an FACSAria II flow cytometer (BD Biosciences) by using FACSDiva software (BD Biosciences). Single cells were expended, as described in the Methods section in this article's Online repository. Positives clones were selected based on their capacity to bind Fel d 1 tetramer compared with unloaded tetramer (empty tetramer).

B-cell and PBMC cultures

B cells were isolated from PBMCs by using an indirect magnetic labeling system (B cell Isolation Kit II, Miltenyi Biotec). These cells were more than 90% $CD19^+$. B cells (2×10^5) were cultured with 10^5 T_H2 clones for 14 days in the presence or absence of the appropriate Fel d 1 peptide in 10% FBS RPMI medium. For culture with the recombinant Fel d 1 protein (40 μg/mL), 2×10^5 purified B cells with or without 10^5 T_H2 clones were cultured in the presence or absence of recombinant Fel d 1 protein (40 μg/mL) with or without 10^5 PBMCs depleted on B cells in 10% FBS RPMI medium. Supernatants were collected after 14 days. Cells were stained after 14 days of culture with appropriate antibodies (see the Methods section in this article's Online Repository).

Statistical analysis

Statistical analysis was performed with the tests indicated in the figure legends by using Prism 5.0 software (GraphPad Software, La Jolla, Calif).

RESULTS

Fel d 1– and Fel d 4–specific T_H2 cells are predominant during cat allergy

Specific T-cell responses to Fel d 1, Fel d 4, Fel d 7, and Fel d 8 were evaluated by using the CD154 upregulation assay, as described in the Methods section (Fig 1, *A*, and see Fig E1 in this article's Online Repository at www.jacionline.org). The CD154 assay accurately measures antigen-specific T cells because a direct comparison of the Fel d 1 CD154 assay and Fel d 1 tetramer assay yielded similar results (see Fig E2 in this article's Online Repository at www.jacionline.org).[18,19] Stimulation of PBMCs from subjects with cat allergy with cat allergen peptides led to detection of high frequencies of memory, but not naive, Fel d 1– and Fel d 4–specific $CD154^+CD4^+$ T cells compared with those in the nonstimulated control and nonallergic subjects (Fig 1, *A* and *B*, and see Figs E3 and E4 in this article's Online Repository at www.jacionline.org). A high T-cell response to Fel d 4 could be found irrespective of a high Fel d 1–specific T-cell response (see Fig E4, *A* and *B*), and there was no correlation between Fel d 1– and Fel d 4–specific T-cell responses (see Fig E4, *C*). Thus the combination of Fel d 1– and Fel d 4–specific T-cell responses covers the majority of the cat allergen–specific T-cell response in allergic subjects (Fig 1, *C*).

The high-frequency Fel d 1– and Fel d 4–specific memory T cells in allergic subjects were found to be chemoattractant