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Chronic cat-allergen exposure induces a T_H2 cell-dependent IgG4 response related to low-sensitization

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Abstract

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 IgG4. However, the specific T cell response that leads to the induction of IgG4 during chronic allergen exposure remains poorly understood.

Objective—To evaluate the relationship between cat allergen-specific T cell frequency, cat allergen-specific IgE and IgG4 titers and clinical status in cat allergic adults with and without cat ownership and the cellular mechanism by which IgG4 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 cells (sT_H2 cells) correlates with IgE level and is linked to asthma. Paradoxically, we observed that cat allergic subjects with chronic cat exposure maintain high frequency of sT_H2 cells, which correlates with IgG4 level and low-sensitization. B cells from allergic, but not from non-allergic subjects, are able to produce IgG4 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 capacity to produce IgG4 are present in allergic subjects; and 2) cat-allergen exposure induces an IgG4 response in a T_H2 cell-dependent manner. Thus, IgG4 accumulation could be mediated by chronic activation of the T_H2 response, which in turn drives desensitization.

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The authors have declared that they have no conflict of interest

Keywords

Cat allergy; Fel d 1; Fel d 4; T_H2 cells; allergen tolerance; asthma; class II tetramer; CD154; IgG4; allergen exposure

Introduction

An allergic reaction is characterized by a type 2 immune response with CD4⁺ T helper 2 (T_H2) cells that produce IL-4, IL-5, IL-13, IL-9 and IL-10, and allergen-specific immunoglobulin E (IgE) that binds to high-affinity IgE receptors (FcεR1) on basophils and mast cells to enhance histamine secretion and the allergic response (1). Although the pathological 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 cat allergy, where a correlation between cat allergen exposure and reduced sensitivity was observed in children with cat ownership (2).

Reduced sensitivity in subjects with cat ownership is associated with the accumulation of specific IgG4 (sIgG4), which has the capacity to attenuate IgE mediated allergic symptoms (3–6). IgG4 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 the induction of sIgG4 (8, 9). Further, induction of IL-10 and sIgG4 is correlated with improved clinical outcome during allergen-specific immunotherapy (10–12). While the importance of these factors in the allergen-tolerance mechanism is therefore evident, the role of T cell subsets and other factors supporting sIgG4 production remain unclear.

Although detection of IgE is routinely used to discriminate allergen sensitivity, characterization of allergen-specific CD4⁺ T cells is more difficult. For cat allergy, the situation is further complicated by the presence of multiple allergens. Fel d 1 is recognized as the major cat allergen and 80–95 % of cat allergic subjects have IgE that recognize Fel d 1 (13); however, IgE towards Fel d 4, Fel d 7 and Fel d 8 are also reported to be present in 60%, 40% and 20% of cat allergic subjects, respectively (14, 15). All of these allergens can be major CD4⁺ T cell targets in cat allergic subjects (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, 18, 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 cat allergic adults with and without cat ownership. The relationship between cat allergen-specific T cell frequency, cat allergen-specific IgE and IgG4 titers and clinical status was examined and the cellular mechanism that led to high IgG4 production in cat allergic subjects was investigated. Here we demonstrate that chronic cat allergen exposure supports a T_H2 cell-dependent IgG4 response.

METHODS

Subjects

38 subjects were recruited from the Virginia Mason Medical Center Allergy Clinic and Benaroya Research Institute. All subjects were HLA typed (Online repository at www.jacionline.org). Subjects with cat allergy (n = 24) had a positive skin test and/or serum IgE response to cat dander greater than 0.35 kU_A/ml using the ImmunoCap test (Phadia AB, Sweden), and had known allergic symptoms. Non-allergic subjects (n = 14) had negative IgE Immunocap scores for cat allergens and other allergens (Dust mite, Bermuda, Timothy grass, Alder and Alternaria). All subjects were recruited with informed consent and institutional review board approval. All assays were performed in a blinded manner with regards to cat ownership status and allergic scores. Asthma and rhinitis scores were based on symptoms and medications as is described in the Online repository at www.jacionline.org.

Ex vivo analysis of cat allergen specific CD4⁺ T cells

For the CD154 expression assay, 30×10⁶ PBMC (at 6×10⁶ /mL) were stimulated for 3h at 37°C with 5µg/mL of synthesized peptide (20 amino acids in length with a 12 amino acid overlap) pools (Mimotopes, 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 DMSO (control), in the presence of 1µg/ml anti-CD40 (HB14, Miltenyi Biotec), in 10% human serum RPMI medium. After stimulation, PBMC were labeled with PE conjugated CD154 and CD154⁺ cells and were enriched using anti-PE magnetic beads (Miltenyi Biotec). A 1/10th fraction of unenriched cells was saved for analysis for frequency determination (Online repository at www.jacionline.org). After enrichment, cells were stained with appropriate antibodies (Online repository at www.jacionline.org). Staining with HLA-DRB1*0301/Fel d 1 chain 2_{17–36} and DRB5*0101/Fel d 1 chain 1_{17–36} 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 (golgi stop, BD Biosciences) according to the manufacturer's instructions. *In vitro* intracellular cytokine staining combined with MHCII tetramer staining was performed as previously described (19). Cells were stained with appropriate antibodies (Online repository at www.jacionline.org).

Specific IgG4 and total IgG4 analysis

The determination of Fel d 1 and Fel d 4 specific IgG4 antibodies in adult serum was performed using ELISA methods described previously (22). Briefly, 1µg/mL recombinant Fel d 1 or Fel d 4 (prepared as previously described (15, 16)) or 1% BSA (as a control) were coated on wells. After washing, 1/10 diluted sera from patients were added to wells coated with Fel d 1 or Fel d 4 or 1% BSA. The detection methods is described in the Online repository at www.jacionline.org. Total IgG4 was quantified in culture supernatant using the Human IgG4 Ready-SET-Go (eBioscience) and following the instructions.

Fel d 1 specific T_H2 clones generation

After staining with DRB5*0101/Fel d 1 chain 1_{17–36} tetramers or DRB1*0301/Fel d 1 chain 2_{17–36}, single memory (CD45RA⁺) tetramer⁺ cells were sorted *ex vivo* in a 96 well plate on an Aria II flow cytometer (BD Biosciences) using FACSDiva software (BD Biosciences). Single cells were expanded as is described in the Online repository at www.jacionline.org. Positives clones were selected on their capacity to bind Fel d 1 tetramer compared to unloaded tetramer (empty tetramer).

B cell and PBMCs cultures

B cells were isolated from PBMCs using an indirect magnetic labeling system (B cell Isolation Kit II, Miltenyi). These cells were more than 90% CD19⁺. 2×10^5 B cells were cultured with 10^5 T_H2 clones for 14 days in presence or absence of the appropriate Fel d 1 peptide, in 10% fetal bovine serum RPMI medium. For culture with the recombinant Fel d 1 protein (40µg/mL), 2×10^5 purified B cells with or without 10^5 T_H2 clones were cultured in presence or absence of recombinant Fel d 1 protein (40µg/mL), with or without 10^5 PBMCs depleted on B cells, in 10% fetal bovine serum RPMI medium. Supernatants were collected after 14 days. Cells were stained after 14 days culture with appropriate antibodies (Online repository at www.jacionline.org).

Statistical analysis

Statistical analysis was performed using the tests indicated in the figure legends, using Prism 5.0 software (GraphPad).

RESULTS

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

Specific T cell responses toward Fel d 1, Fel d 4, Fel d 7 and Fel d 8 were evaluated by using the CD154 up-regulation assay as described in Materials and Methods (Fig 1A and Fig E1). The CD154 assay accurately measures antigen-specific T cells as a direct comparison of the Fel d 1-CD154 assay and Fel d 1-tetramer assay yields similar results (18, 19) (Fig E2). Stimulation of PBMCs from cat allergic subjects with cat allergen peptides led to detection of high frequencies of memory, but not naïve, Fel d 1 and Fel d 4 specific CD154⁺ CD4⁺ T cells compared to the non-stimulated control and to non-allergic subjects (Fig 1A–B, Fig E3 and E4). A high T cell response to Fel d 4 could be found irrespective of a high Fel d 1 specific T cell response (Fig E4A and B) and, indeed, there was no correlation between Fel d 1 and Fel d 4 specific T cell responses (Fig E4C). Thus, the combination of Fel d 1 and Fel d 4 specific T cell responses cover the majority of the cat allergen specific T cell response in allergic subjects (Fig 1C).

The high frequency Fel d 1 and Fel d 4 specific memory T cells in allergic subjects were found to be CRT_H2⁺ and CD27⁺ T cells and correspond to an effector T_H2 phenotype (17, 23) (Fig 1D–E). Moreover, accumulation of allergen-specific memory CD4⁺ T cells is directly linked to the effector T_H2 phenotype (Fig E5). A relatively high frequency of Fel d 7 specific memory CD4⁺ T_H2 cells (sT_H2 cells) was also observed in two out of 24 subjects

examined, (Fig E4 and E5) but the overall response of the group was not significantly different.

Cat exposure correlates with low-sensitization and a relatively high frequency of allergen specific CD4⁺ T_H2 cells in allergic subjects

Nine allergic adults in this study were cat owners and were therefore constantly exposed to cat allergens (Table 1). We found that allergic adults with cat ownership and allergic adults without a cat at home had similar cat dander specific IgE (sIgE) levels (Table 2); however, cat owners had significantly lower skin prick test scores (Table 2). Cat owning allergic adults also had significantly lower rhinitis symptom severity (78% with mild symptoms and 22% with moderate symptoms) than allergic adults without a cat (13% with mild symptoms, 73% with moderate symptoms and 6% with severe symptoms). However, regarding asthma prevalence, no significant difference were observed between these two allergic groups.

In this study, cat owning allergic adults had significantly higher levels of serum Fel d 1 and Fel d 4 specific IgG4 (sIgG4) than non-allergic adults with or without a cat or allergic adults without a cat (Fig 2A and Fig E6). Moreover, the sIgG4/sIgE ratio was significantly higher in allergic adults who own cat compared to allergic adults who did not own cat (Fig 2B). Allergic adults with cat ownership also had a higher frequency of Fel d 1 and Fel d 4 sT_H2 cells than non-allergic adults with or without a cat (Fig 2C and Fig E6). Allergic cat owners also had a higher frequency of Fel d 1 and Fel d 4 T cells compared to allergic subjects without cat, though the difference was not statistical significant. However, the IgG4 accumulation observed in cat owning allergic adults was not associated with a shift of T_H2 cells to CXCR5⁺ T follicular helper (T_{FH}) cells that are known to support IgG production (24, 25) (Fig E7).

The observed T_H2 phenotype of cat allergen-specific T cells in allergic adults with cat ownership (Fig 2C) is supported by *ex vivo* and *in vitro* cytokine secretion, in which Fel d 1 specific memory CD4⁺ T cells primarily secreted IL-4 and IL-5. No difference in cytokine profile of sT_H2 cells was observed between allergic groups (Fig 2D–G).

Specific T_H2 cell response correlates with the allergen immunoglobulin response

In non-cat owning allergic adults, sT_H2 cell frequency correlated with cat dander sIgE levels but not with sIgG4 levels (Fig 3A–B). In contrast, in cat owning allergic adults the sT_H2 frequency correlated with sIgG4 but not with sIgE (Fig 3A–B). In our non-cat owning allergic cohort, we observed a link between asthma prevalence, sT_H2 cell frequency and sIgE levels (Fig 3C). In contrast, there was no link between asthma and sT_H2 cell frequency or IgE levels in cat owning allergic subjects (Fig 3D). These results demonstrate that cat allergen exposure modulates the relationship between the T_H2 response, sIgE level and asthmatic prevalence. Moreover, these results suggest a link between the T_H2 response and IgG4 accumulation for cat allergic subjects with cat ownership.

Fel d 1 specific T_H2 cells support IgG4 production by B cells from allergic subjects

We tested the hypothesis that T_H2 cells can support IgG4 production. For evaluation of the capacity of T_H2 cell to induce IgG4 in absence of chronic allergen exposure, single-cell

sorting and cloning of Fel d 1 tetramer positive memory cells were performed to generate Fel d 1 sT_H2 clones from allergic subjects without cat ownership (Fig E8). Fel d 1 sT_H2 clones were cultured with isolated B cells from allergic (with or without cat ownership) and non-allergic subjects without cat ownership (with matching HLA to the T_H2 clones used in the experiment), in presence of the appropriate Fel d 1 peptide to induce a cognate T-B cell response (Fig 4). After 14 days of T and B cell co-culture, total IgG4 was detected in cultures with sT_H2 clones and B cells from allergic adults (Fig 4A and B) but not in those with B cells from non-allergic adults (Fig 4C). CD38 expression was upregulated on the surface of sT_H2 clones and B cells in the presence of specific peptide in cultures with B cells from both allergic and non-allergic subjects (Fig 4D–F and Fig E9), demonstrating the activation of these cells during the cognate immune response.

Because chronic allergen exposure can influence T cell function, we also test the ability of sT_H2 clones from allergic subjects with cat ownership to induce IgG4. In accordance with previous observation (Fig 2D–G), sT_H2 clones from allergic subjects with and without cat ownership had similar T_H2 cytokine profile (Fig E10). Moreover, sT_H2 clones from allergic subjects with cat ownership had similar capacity to induce IgG4 production by B cells compare to sT_H2 clones from allergic subjects without cat ownership (Fig E11).

These experiments demonstrate the capacity of T_H2 cells to support IgG4 production by B cells of allergic subjects, but not those of non-allergic subjects.

The Fel d 1 recombinant protein enhances IgG4 production in a T_H2 cell dependent manner

Asthmatic status and chronic allergen exposure are both linked to a T_H2 response (Fig 2 and 3); however, the immunoglobulin response under these two conditions is different as an increase in sIgG4 is observed only in the chronic allergen exposure group. We therefore speculated a direct role for Fel d 1 protein in inducing IgG4 production in cat allergic subjects.

B cells from allergic subjects had already undergone an IgG4 class switch and are potentially allergen-experienced (Fig 4), we tested their capacity to produce IgG4 upon Fel d 1 recombinant protein activation. B cells isolated from allergic subjects without cat ownership were capable of producing IgG4 in response to Fel d 1 only in presence of sT_H2 clones (Fig 5A). These results suggest that T_H2 cells support IgG4 production through direct interaction with allergen-experienced B cells after they encounter the allergen.

On the other hand, B cells from non-allergic subjects were incapable of producing IgG4 in the presence of sT_H2 clones and Fel d 1 protein (Fig 5B). Only concomitant culture of B cells of non-allergic subjects with Fel d 1, sT_H2 clones, and antigen presenting cells (APCs = PBMCs depleted of B cells) was sufficient to induce IgG4 (Fig 5C). These experiments were Fel d 1 specific as IgG4 was not induced in the presence of Fel d 4 (40 µg/mL) (Fig E12). These experiments suggest that T_H2 cells cooperate with cells from the innate immune system to induce IgG4 class switch.

DISCUSSION

The cat allergen-specific T cell response is characterized by a higher frequency of Fel d 1 and Fel d 4 sT_H2 cells in allergic subjects than in non-allergic subjects. High frequency of Fel d 7 specific memory T_H2 CD4⁺ T cell responses were only observed in approximately 10% of our cohort. A correlation between frequency of Fel d 1 & Fel d 4 sT_H2 cells and IgE level was observed in the group without a cat at home. Higher frequency of sT_H2 cells is also linked to increase in asthma prevalence in subjects without cat ownership. These correlations were not observed in the group with cat ownership and the frequency of Fel d 1 & Fel d 4 sT_H2 cells correlated only with sIgG4, and is linked to low sensitization. At the T cell level, we did not observed difference in cytokine profiles between these two groups. However, the possibility of molecular modifications of sT_H2 cells during chronic cat allergen exposure remain to be elucidated.

Earlier work supports that both IL-10 and IL-4 regulate IgG4 production (26, 27). IgG4 is linked to decreased sensitivity, but IgG4 is also known to correlate with cat allergen exposure in cat allergic subjects (28–30). In accordance with this, we observed low allergen-sensitivity in cat allergic subjects with a cat at home. We also observed a direct correlation between sT_H2 cell frequency and sIgG4 level for this group of subjects. Moreover, we provide evidence that the cognate response (using peptide or whole protein) between Feld 1 sT_H2 clones and B cells from allergic subjects is sufficient to induce IgG4 production. This response may be mediated by allergen-experienced B cells after antigen recall, which have a capacity to rapidly differentiate into plasma cells (31). These results demonstrate the direct role of T_H2 cells in support of IgG4 production and suggest that an IgG4 response is part of a T_H2 driven response upon allergen exposure. We speculate the repeated stimulation of B cells by allergens and T_H2 cells could explain the accumulation of IgG4 during allergen exposure without major modification on the T_H2 response. We observed IgG4 accumulation upon allergen exposure is related and dependent of the presence of T_H2 cells. However, T_H2 cells *in vivo*, in absence of allergen exposure, does not seem to have the capacity to mediate IgG4 accumulation, indicating a major role of repeated allergen exposure in this phenomenon.

On the other hand, T_H2 cells were not able to directly induce an IgG4 class switch on B cells from non-allergic subjects (allergen-unexperienced B cells), implicating multiple signaling requirements for B cells to induce IgG4 class switching. The immunoglobulin class switch can be induced by T-cell dependent and T-cell independent signals (32–35). For allergen-unexperienced B cells from non-allergic subjects, activation of the innate response by Fel d 1 is essential for the T_H2 cell-dependent IgG4 production. This phenomenon may underlie the primary induction of IgG4 after initial encounter with allergen. While the innate response alone is not sufficient to induce an IgG4 class switch, innate cells still play an important role in the T-cell dependent immunoglobulin class switch.

The ability of some allergens to directly activate innate cells is already known (36, 37). Thus, this phenomenon as observed here likely applies to other allergens like Fel d 4, and indeed has been described for the protein Can f 6 (a lipocalin protein similar to Fel d 4), which can also activate innate cells (14, 36, 37).

The major T-cell dependent immunoglobulin response previously described is mediated by T_{FH} cells (31, 34, 38); however, in this study we were unable to detect CXCR5 expression by sT_{H2} cells, suggesting T_{FH} cells are not involved in sIgG4 production upon allergen exposure. Moreover, *ex vivo* assay of human sera reveal an acute accumulation of IgG4 only during exposure (28, 30). These observations suggest IgG4 is not produced by long-lived plasma cells and is independent of the germinal center pathway. Transient production of IgG4 can be explained by differentiation of IgG4-B cells into short-lived extrafollicular plasma cells localized in the marginal zone (39).

This work provides new insights into the anti-inflammatory role of IgG4 at the cellular level and how chronic allergen exposure can modulate the immunoglobulin response. IgG4 accumulation has previously been observed during chronic parasite infection, where it led to immunoregulation and a hyporesponse (40–42). These observations, combined with the results described here, suggest that chronic activation of T_{H2} cells and B cells (by allergen or parasites) mediates IgG4 accumulation.

The phenomenon we describe here may also occur during allergen-immunotherapy, where an increase in sIgG4 could mediate the decrease of allergic symptoms without induction of T cell tolerance. Indeed, IgG4 accumulation during allergen-immunotherapy is correlated with a decrease in allergic symptoms (10, 11, 43–45).

In summary, both asthmatic status and cat allergen exposure are linked to a high frequency of T_{H2} cells. We further demonstrate that during chronic cat allergen exposure, sT_{H2} cells support IgG4 production by allergen-experienced B cells, which in turn drives desensitization.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

CRT_{H2}	Chemoattractant receptor-homologous molecule expressed on T _{H2} cells
HLA	Human Leukocyte Antigen

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Key Messages

- Chronic cat allergen exposure is related to low-sensitization.
- Correlation between specific T_H2 cells accumulation and IgG4 level during chronic cat allergen exposure was observed.
- Specific T_H2 cells support IgG4 production by allergen-experienced B cells in response to Fel d 1.

FIG 1. Fel d 1 and Fel d 4 specific memory T_{H2} cells are predominant during cat allergy
 (A) CD154 expression after stimulation with cat allergen peptides or DMSO (none). Numbers indicate frequency of memory CD45RA⁻ CD154⁺ T cells per 10⁶ CD4⁺ T cells.
 (B–C) Frequency of Fel d 1, Fel d 4, Fel d 7 and Fel d 8 (B) and of combined Fel d 1 and Fel d 4 (C) memory CD154⁺ T cells after cat allergen peptide stimulation. (D) CRT_{H2}, CD27, CCR4, CXCR3 and CD154 expression on gated memory Fel d 1 or Fel d 4 specific T cells from 2 different allergic adults. The percentage of each marker on CD154⁺ T cells is as indicated. (E) Frequency comparison of combined Fel d 1 and Fel d 4 specific memory

CD154⁺ CRT_H2⁺ (top) or CD27⁻ (bottom) T cells between allergic and non-allergic subjects. Data were compared using Mann Whitney test (*: p 0.05, **: p 0.01, ***: p 0.001).

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FIG 2. Cat allergen exposure induces sIgG4 and allergen specific CD4⁺ T_H2 cell response in allergic adults

(A) Combined Fel d 1 and Fel d 4 specific IgG4 levels in the serum of non-allergic and allergic adults. (B) sIgG4/sIgE ratio (log-transformed) in cat allergic subjects with and without cat ownership. (C) Combined frequency of Fel d 1 and Fel d 4 specific memory CD45RA⁻, CRT_H2⁺, or CD27⁻ CD154⁺ T cells. (D) CD154 and cytokine expression on gated CD4⁺ T cells after Fel d 1 peptide stimulation. (E) Comparison of cytokine expression of CD154⁺ T cells in allergic adults. (F) Intracellular cytokine staining of DRB5 restricted Fel d 1 chain 117–36 specific CD4⁺ T cells in a DRB5 cat owning allergic adult, using tetramer staining. Percentages of IL-4⁺, IL-5⁺, IL-17⁺, IFN- γ ⁺ and IL-10⁺ Fel d 1 specific CD4⁺ T cells are indicated. (G) Cytokine expression of tetramer positive Fel d 1 specific CD4⁺ T cells in allergic adults. Data were compared using student's unpaired t-test (B) or Kruskal-Wallis test with Dunn's multiple comparisons test (A and C) or Mann Whitney test (E and G) (*: p 0.05, **: p 0.01, ***: p 0.001).

FIG 3. The correlation between the T_H2 cell response and sIgE or sIgG4 level depends on allergen exposure

(A–B) Correlation between the combined frequency of Fel d 1 and Fel d 4 specific memory $CD154^+ CRT_{H2}^+ CD45RA^- CD4^+$ T cells and the total cat dander specific IgE level (A) or the combined Fel d 1 and Fel d 4 specific IgG4 level (B) in allergic adults with or without cat. (C–D) Comparison of combined frequency of Fel d 1 and Fel d 4 specific memory $CD154^+ CRT_{H2}^+$ or $CD27^-$ and sIgE between asthmatic and non-asthmatic allergic adults without (C) or with cat (D). Spearman R and P values were determined to analyze the correlation. Data were compared using Mann Whitney test (**: $p < 0.01$).

FIG 4. Fel d 1 specific T_H2 cells support IgG4 production by allergen-experienced B cells
 (A–C) Total IgG4 in the culture supernatant of B cells from DRB5 and DR3 non-allergic (3 subjects, 10 clones) (C) and allergic subjects without cat ownership (3 subjects, 14 clones) (A) or with cat ownership (3 subjects, 16 clones) (B), in the presence or absence of Fel d 1 chain 1_{17–36} or Fel d 1 chain 2_{17–36} specific CD4⁺ T clones from DRB5 or DR3 allergic subjects without cat, with or without the Fel d 1 chain 1_{17–36} or Fel d 1 chain 2_{17–36} peptide. (ND = non detectable). (D–E) CD38 expression on B cells were shown in bottom panels. Each clone were tested on duplicate or triplicate wells and minimum of 4 experiments were done per group. Data were compared using Mann Whitney test (**: p 0.01, ***: p 0.001).

FIG 5. T_H2 cell-dependent IgG4 production in response to Fel d 1 recombinant protein
Total IgG4 in culture supernatant of B cells isolated from DRB5 and DR3 allergic (3 subjects, 11 clones) (A) or non-allergic (3 subjects, 12 clones) (B and C) subjects, with or without CD4⁺ T_H2 clones and Fel d 1 recombinant protein (40μg/mL), and with autologous PBMCs depleted of B cells (APC) (C). Each clone were tested on duplicate or triplicate wells and minimum of 4 experiments were done per group. Data were compared using Mann Whitney test (A–D) (*: p < 0.05).

Table 1

Subject Information

Samples n	HLA DR	Asthma	Rhinitis score	cat owner	age of first ownership	current age	SPT	IgE (kU/L)
allergy#1	404 1302	yes	mild	no		36	3	6.35
allergy#2	301 1501	yes	moderate	no		32	4	23.6
allergy#3	701 1501	yes	mild	no		32	4	3.91
allergy#4	901 1501	yes	severe	no		42	4	33.8
allergy#5	401 701	yes	moderate	no		37	3	72.3
allergy#6	301 401	yes	N/A	no		28	4	7.59
allergy#7	1401 404	yes	moderate	no		66	N/A	25
allergy#8	10** 405	yes	moderate	no		34	4	20.8
allergy#9	1501 1301	yes	moderate	no		35	3	4.94
allergy#10	1101 1501	no	moderate	no		54	4	0.49
allergy#11	301 401	no	moderate	no		61	4	5.65
allergy#12	1001 1404	no	moderate	no		39	4	1.28
allergy#13	1101 1502	no	moderate	no		30	4	4.42
allergy#14	101 1501	no	moderate	no		46	4	0.96
allergy#15	1101 1501	no	moderate	no		57	4	3.88
allergy#16	401 1301	yes	mild	yes	17	48	4	3.59
allergy#17	301 401	yes	mild	yes	8	46	3	12.5
allergy#18	301 801	yes	mild	yes	6	50	2	6.42
allergy#19	701 1301	yes	moderate	yes	0	36	2	58.3
allergy#20	701 1201	yes	moderate	yes	0	67	4	7.1
allergy#21	301 407	no	mild	yes	0	34	2	9.03
allergy#22	701 1101	no	mild	yes	3	39	3	11.4
allergy#23	1501 07**	no	mild	yes	5	36	2	21.3
allergy#24	401 1101	no	mild	yes	3	32	3	8.41
Control#1	701 1302	-		no		42	N/A	0
Control#2	701 801	-		no		32	N/A	0

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Samples n	HLA DR	Asthma	Rhinitis score	cat owner	age of first ownership	current age	SPT	IgE (kU/L)
Control#3	1104	1104	-	no		30	N/A	0
Control#4	407	102	-	no		30	N/A	0
Control#5	401	03**	-	no		30	N/A	0
Control#6	701	1302	-	no		34	N/A	0
Control#7	106	1501	-	no		30	N/A	0
Control#8	1501	1501	-	no		32	N/A	0
Control#9	301	1104	-	yes	29	33	N/A	0
Control#10	301	401	-	yes	26	37	N/A	0
Control#11	404	1601	-	yes	0	31	N/A	0
Control#12	404	1301	-	yes	7.5	66	N/A	0
Control#13	401	1501	-	yes	20	28	N/A	0
Control#14	101	103	-	yes	0	34	N/A	0

N/A = not available; SPT = Skin Prick Test (0 to 4+)

Table 2

Influence of cat exposure on sensitization, IgE, and clinical symptoms.

	non allergic	Allergic		p value
		cat owner	without cat	
number of samples (n)	14	9	15	
Skin Prick Test (0 to 4+)	ND	2.8 ± 0.3	3.8 ± 0.1	0.0009*
IgE (kU/L)	0	15.3 ± 5.6	14.3 ± 4.9	0.8974*
no asthma	-	44.5% (n=4)	40% (n=6)	
asthma	-	55.5% (n=5)	60% (n=9)	
odds (yes/no)		1.25	1.5	1§
mild rhinitis	0	78% (n=7)	13% (n=2)	
moderate rhinitis	0	22% (n=2)	73% (n=11)	
severe rhinitis	0	0	6% (n=1)	
odds (moderate or severe/mild)		0.3	6	0.0066§

* student's unpaired t-test;

§Fisher exact test