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Ara h 1-Reactive T Cells in Peanut Allergic Individuals

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Abstract

Background—Effective immunotherapy for peanut allergy is hampered by a lack of understanding of peanut-reactive CD4⁺ T cells

Objective—To identify, characterize and track Ara h 1-reactive cells in peanut allergic subjects using Ara h 1-specific class II tetramers.

Methods—Tetramer Guided Epitope Mapping (TGEM) was used to identify the antigenic peptides within the peanut allergen Ara h 1. Subsequently, HLA class II/Ara h 1-specific tetramers were used to determine the frequency and phenotype of Ara h 1-reactive T cells in peanut-allergic subjects. Cytokine profiles of Ara h 1-reactive T cells were also determined.

Results—Multiple Ara h 1 epitopes with defined HLA restriction were identified. Ara h 1-specific CD4⁺ T cells were detected in all of the peanut-allergic subjects tested. Ara h 1-reactive T cells in allergic subjects expressed CCR4 but did not express CRTH2. The percentage of Ara h 1-reactive cells that expressed the β 7 integrin was low compared to total CD4⁺ T cells. Ara h 1-reactive cells that secreted IFN- γ , IL-4, IL-5, IL-10 and IL-17 were detected.

Conclusions—In peanut-allergic individuals, Ara h 1-reactive T cells occurred at moderate frequencies, were predominantly CCR4⁺ memory cells and produced IL-4. Class II tetramers can be readily used to detect Ara h 1-reactive T cells in the peripheral blood of peanut allergic subjects without *in vitro* expansion and would be effective for tracking peanut-reactive CD4⁺ T cells during immunotherapy.

Keywords

Food allergy; Peanut; Ara h 1; T cells; Class II tetramers

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Capsule Summary

Peanut-specific CD4⁺ T cell frequencies and phenotypes were measured directly *ex vivo* through new advances in class II tetramer technology. The use of tetramers will facilitate the interrogation of peanut-specific T cells in clinical trials.

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INTRODUCTION

Peanut allergy is relatively common, affecting approximately 1% of children in the U.S. and UK. Peanut sensitivity typically presents early in life, but is often permanent, as only 20% of young children resolve their food allergy by adulthood ^(1;2). In addition, peanut allergy is associated with a significant risk of fatality. Currently, the only standard treatment options for peanut allergy sufferers are vigilant avoidance and administration of epinephrine in the event of an accidental ingestion. Both of these are associated with considerable psychological morbidity. As a consequence, multiple approaches to induce tolerance to peanut have been carried out. Studies initiated more than 15 years ago demonstrated that desensitization is possible for peanut allergy by injection of crude peanut extract ^(3;4). However these injections were associated with frequent episodes of anaphylaxis ⁽⁵⁾. More recently, peanut oral immunotherapy trials were attempted, and the outcomes were encouraging ⁽⁶⁻⁸⁾. However, even in these recent oral immunotherapy trials, treatment was associated with side effects that required medication ⁽⁶⁾.

Peanut-specific CD4+ T cells are known to be involved in the pathophysiology of peanut allergy and could act as a useful biomarker for evaluating the progress of peanut oral immunotherapy ^(9;10). Ideally, a T cell biomarker should distinguish between desensitization and tolerance induction. It would also be useful to predict the likelihood of relapse after induction of tolerance. In response to the need of a reliable biomarker for monitoring, this present study sought to develop and apply class II tetramer reagents to monitor peanut-reactive T cells. Identification of T cell epitopes for the common peanut allergens is crucial for the development of such reagents. At present, 11 peanut allergens have been identified (<http://www.allergen.org>). Sensitization to Ara h 1 and Ara h 2 has been found in 80% to 95% of North American peanut-allergic patients ^(11;12). However, sensitization to Ara h 1 seems to be lower in peanut allergic subjects in European countries, ranging from 30% to 75% ⁽¹²⁻¹⁴⁾. Sensitization to Ara h 6 was high in some European populations and sensitization to Ara h 9 was found to be the most prevalent in Mediterranean populations ^(12;13). Multiple IgE epitopes have been identified in Ara h 1 ⁽¹⁵⁾, but no primary data have been published on Ara h 1 T cell epitopes. In this present study, we first used Tetramer Guided Epitope Mapping to identify CD4+ T cell epitopes in Ara h 1, and subsequently used these class II tetramer reagents to study CD4+ T cells specific for Ara h 1 in peanut-allergic and non-allergic individuals. Ara h 1 T cell epitopes restricted by eight HLA class II alleles were identified. Using tetramers corresponding to these epitopes, we examined the frequency and phenotype of peanut-specific CD4+ T cells in allergic and non-allergic individuals. In addition, cytokine analysis was performed on Ara h 1-specific T cell clones and lines from peanut-allergic individuals.

METHODS

Human subjects

Subjects were recruited from the Virginia Mason Medical Center Allergy Clinic and Benaroya Research Institute. Allergic subjects with a documented record of peanut anaphylaxis and positive ImmunoCap scores for peanut-specific IgE were recruited. A subset of these subjects also had documented seasonal allergy. Atopic subjects without peanut allergy and non-atopic subjects with no clinical symptoms of allergy and negative ImmunoCap scores for Timothy grass pollen, cat, dust mite and peanut were also recruited. DNA samples were HLA typed using Dynal Unitray SSP Kits (Invitrogen) according to manufacturer's instructions. All subjects were recruited with informed consent and IRB approval. A summary of the attributes of these human subjects is shown in Table 1.

Tetramer Guided Epitope Mapping

Biotinylated HLA-DR proteins were purified as described⁽¹⁶⁾. A total of 77 peptides (p1 to p77), which were 20 amino acids in length with a 12 amino acid overlap spanning the entire Ara h 1 sequence (including the signal peptide), were synthesized (Mimotopes, Clayton Australia). These peptides were divided into 14 pools of 5 peptides each plus a 15th pool of 7 peptides. These peptide mixtures were loaded into the biotinylated HLA-DR proteins to generate pooled tetramers as described⁽¹⁷⁾. Cells were cultured for 14 days and then stained with pooled peptide tetramers. Cells from wells which gave positive staining were stained again using tetramers loaded with each individual peptide from the positive pool.

Ex vivo analysis of Ara h 1 and pollen reactive CD4+ T cells

The frequency of Ara h 1-specific T cells was measured as previously described^(18;19). Briefly, 30 million PBMC in 200 µl of T cell culture medium were stained with 20 µg/ml PE-labeled tetramers at room temperature for 100 min. Cells were then stained with anti-CD3 FITC (eBioscience), anti-CD4 APC (eBioscience), anti-CD14 PerCP (BD Pharmingen) and anti-CD19 PerCP (BD Pharmingen) for 20 minutes at 4°C. Cells were washed and incubated with anti-PE magnetic beads (Miltenyi Biotec) at 4°C for 20 minutes, washed again, and a 1/100th fraction saved for analysis. The other fraction was passed through a magnetic column (Miltenyi Biotec). Bound, PE-labeled cells were flushed and collected. Cells in the bound and pre-column fractions were stained with Via-Probe (BD Bioscience) for 10 minutes before flow cytometry. Data were analyzed using FlowJo (Tree Star), gating on FSC/SSC and excluding CD14+ and CD19+ populations and Via-Probe+ (dead) cells. Frequency was calculated as previously described⁽¹⁹⁾.

For phenotyping studies, antibodies against markers of interest were used instead of anti-CD3. Staining for Aln g 1 and Phl p 1 reactive T cells was carried out with DR0701/Aln g 1₁₃₇₋₁₅₆, DR1501/Aln g 1₁₃₇₋₁₅₆ and DR0401/Phl p 1₁₂₀₋₁₃₉ tetramers.

Intracellular cytokine staining

For intracellular staining of IFN-γ, IL-4, IL-17, IL-5, and/or IL-10, PBMCs were stimulated for 2 weeks with specific peptide, and then stained with the corresponding PE-labeled tetramers for 60 minutes at 37°C. Cells were then re-stimulated with 50ng/mL PMA and 1µg/mL ionomycin in the presence of 10µg/ml monensin in 1 ml of complete media for 6 hours at 37°C, 5% CO₂. Following re-stimulation, cells were stained with anti-CD4 (BD Pharmingen), anti-CD3 (eBioscience), and a combination of anti-CD14 (BD Pharmingen), anti-CD19 (Dako) and ViViD reagent (Invitrogen) to exclude non-specific tetramer staining. After 30 minutes at room temperature, cells were fixed with fixation buffer (eBioscience), washed twice with a permeabilization buffer (eBioscience), and stained in 200 µl permeabilization buffer with various combinations of anti-IFN-γ, anti-IL-17, anti-IL-10 (all from Biolegend), anti-IL-4 (eBioscience) and anti-IL-5 (Miltenyi Biotec). After 30 minutes at 4°C, cells were washed and immediately analyzed by flow cytometry.

RESULTS

Identification of CD4+ T cell epitopes in Ara h 1

A total of twelve peanut-allergic subjects with a history of anaphylaxis to peanut were recruited for this study. Six non-atopic subjects and 5 atopic subjects without peanut allergy were also recruited as control subjects. The Tetramer Guided Epitope Mapping approach was used to identify CD4+ T cell epitopes for Ara h 1 in peanut-allergic subjects as described in the methods section. Detailed results for one of these experiments with a representative DR1101 subject are shown in Supplementary Figure E1. Ara h1₁₆₉₋₁₈₈, Ara h1₃₂₁₋₃₄₀, Ara h1₄₅₇₋₄₇₆ and Ara h1₄₆₅₋₄₈₄ were identified as DR1101-restricted T cell

epitopes. A complete summary of results from all epitope identification experiments is shown in Table 2. In summary, a total of 20 epitopes were identified, restricted by DR0101, DR0301, DR0401, DR0404, DR1101, DR1401, DR1502 and DRB5. It is likely that pairs of consecutive peptides, such as Ara h 1₄₅₇₋₄₇₆/Ara h 1₄₆₅₋₄₈₄ (restricted by DR1101) and Ara h 1₃₂₁₋₃₄₀/Ara h 1₃₂₉₋₃₄₈ (restricted by DR1401) contain an identical minimum epitope. In using TGEM, we failed to identify any Ara h 1 epitopes restricted by DR0701 and DR1501. However, each subject with a DR0701 or DR1501 haplotype recognized Ara h 1 epitopes restricted by another class II allele. Therefore it is possible that there are no DR1501 and DR0701 restricted Ara h 1 epitopes. Alternatively, DR0701 and DR1501 restricted Ara h 1 specific T cells may be of low avidity, making them difficult to detect using tetramers.

Ex vivo detection and cell surface phenotyping of Ara h 1-specific CD4+ T cells

In order to detect Ara h 1-specific T cells directly *ex vivo*, we used anti-PE magnetic beads to enrich for PE-labeled tetramer-positive cells. This approach enabled us to characterize the phenotype and frequency of Ara h 1-reactive T cells in the peripheral blood without expanding PBMC *in vitro*. Representative results for a DR1101 peanut-allergic subject and a DR1101 non-atopic subject are shown in Figure 1A. The results of additional experiments are summarized in Figure 1B. These results indicated that the average frequency of Ara h 1-reactive T cells in peanut-allergic subjects was approximately 9 cells per million while the average frequency in non-atopic subjects and atopic subjects without peanut allergy was less than 1 cell per million. *Ex vivo* tetramer staining of Ara h 1-reactive T cells also allowed direct examination of cell surface phenotypes for Ara h 1-reactive T cells in allergic subjects, using surface markers such as CD45RA (a naïve T cell marker), CD45RO (a memory T cell marker), CRTh2 and CCR4 (Th2 markers) ^(20;21), and CLA and β7 integrin (T cell homing markers) ^(22;23). The expression of each of these markers on Ara h 1-reactive T cells was compared to that of total CD4+ T cells. Representative results for one of these experiments are shown in Figure 2A. Complete results from multiple subjects are summarized in Figure 2B. In total, the data indicate that Ara h 1-reactive T cells in peanut allergic subjects were memory T cells and expressed the Th2 marker CCR4. The majority of these cells did not express CRTh2, and only a small fraction of these cells expressed the gut homing marker β7 integrin. The majority of Ara h 1-reactive T cells also expressed CD25. The frequency of Ara h 1-reactive T cells in non-allergic subjects was very low, which precluded the examination of their phenotypes. As indicated in Table 1, three peanut-allergic subjects also had a seasonal allergy to Timothy grass or alder pollen. Since we had also developed appropriate tetramer reagents to study these pollen reactive T cells, we examined the phenotype of pollen-specific T cells in these peanut allergic subjects. This allowed a comparison of Ara h 1-reactive T cells with Aln g 1 (Alder pollen allergen) or Phl p 1 (Timothy grass pollen allergen) reactive T cells within the same subjects. As shown in Supplementary Figure E2, the results of these experiments indicated that while the majority of Ara h 1-, Aln g 1- and Phl p 1-reactive T cells expressed CCR4, only Aln g 1- and Phl p 1-reactive T cells expressed CRTh2.

Cytokine profiles of Ara h 1-reactive T cells

The CCR4 surface phenotype of Ara h 1-reactive T cells indicated that these T cells belong to the Th2 lineage. The Th2 phenotype of these cells was further confirmed by examining the cytokine profiles of Ara h 1-specific T cell lines and clones derived from peanut-allergic subjects. Ara h 1-specific cell lines were generated by stimulating the PBMC of peanut allergic subjects with antigenic Ara h 1 peptides for two weeks. Ara h 1-reactive T cell clones were isolated by sorting single Ara h 1 tetramer-positive T cells from Ara h 1 lines and subsequently expanding them with PHA. Cytokine profiles were examined by tetramer staining in conjunction with intra-cellular cytokine staining (ICS). Representative results of these assays are shown in Figure 3. Additional data for multiple subjects are summarized in

Table 3. We observed that all of the Ara h 1-reactive cell lines and clones examined produced IL-4. At least one third of the lines also produced IL-5. More than half of the cell lines produced a low amount of IFN- γ . As shown in Figure 4, multicolor ICS identified cell lines that produced IL-4 and IL-5 simultaneously or IL-4 individually. Cell lines that produced IL-10 or IL-17 individually or in combination with IL-4 were also observed (Figure 4), though the percentage of IL-4 and IL-17 co-producers was minimal. Release of IL-5 and IL-13 by Ara h 1-specific lines was confirmed by measuring cytokine in the supernatants of our Ara h 1-stimulated T cell lines using the MesoScale Discovery multiplex kit (Supplementary Figure E3). These experiments indicated that T cell lines stimulated with Ara h 1 peptides produced at least 8 fold more IL-5 and IL-13 than IFN- γ . In total, these data indicated that the majority of Ara h 1-reactive CD4⁺ T cells in peanut-allergic subjects were Th2 cells, but also confirmed the existence of Ara h 1-reactive cells that produced IFN- γ , IL-10 and IL-17.

DISCUSSION

While basic and clinical research have been ongoing for a number of years, CD4⁺ T cells specific for food allergens remain poorly characterized. In particular, the phenotype of these cells has not been examined *ex vivo*. Therefore it is unknown whether food allergen-specific cells differ from other allergen specific CD4⁺ T cells. In this study, we developed class II tetramer reagents to identify CD4⁺ epitopes for a major peanut allergen, Ara h 1. We further utilized these tetramer reagents to directly examine the frequency and phenotype of Ara h 1-reactive T cells in adult subjects that had a documented history of peanut anaphylaxis. Ara h 1-specific T cells could be readily detected in the PBMC of peanut allergic subjects. However, their mean frequency (approximately 9 cells per million) was slightly lower than to the frequency of alder or Timothy grass pollen-specific T cells within the same subjects (data not shown). Using a similar approach, Ara h 1 epitope-specific T cells were barely detectable in subjects without peanut allergy (the average frequency was less than 1 cell per million). The scarcity of detectable Ara h 1-reactive T cells in the majority of peanut non-allergic subjects suggests that these cells exist at low frequencies. Alternatively, it is possible that Ara h 1-reactive T cells in non-allergic subjects are of low avidity and therefore difficult to detect using tetramers⁽²⁴⁾. Using ICS assays, Prussin et al reported that the frequency of peanut-reactive T cells in peanut-allergic subjects was 100 to 200 per million CD4⁺ T cells⁽²⁵⁾. The frequencies observed here were 10-fold lower. However, much of this discrepancy can be explained by differences in methodology. The frequencies observed in this report we determined for individual antigenic epitopes from a single peanut protein restricted by a single HLA. In contrast, Prussin et al stimulated with peanut extract, which has multiple peanut allergens, each containing multiple epitopes. In addition, most subjects have T cells restricted by multiple class II alleles. Taking these factors into account, the frequency of peanut epitope-specific CD4⁺ T cells seems comparable for these two studies.

All of the peanut-allergic subjects recruited for this study had a history of anaphylactic reaction to peanut. Given this severity of disease, it could be considered surprising that the frequency of Ara h 1 epitope-specific T cells was lower than pollen epitope-reactive T cells in the same subjects. It could be argued that a long period of peanut avoidance for this group of subjects may have reduced the frequency of Ara h 1-reactive T cells. In support of this notion, it has been previously observed that the frequency of pollen-reactive T cells can be several-fold lower out of the pollen season than within the peak pollen season⁽²⁶⁾. Alternatively, it could be that the most frequent peanut-reactive T cells are directed toward peanut allergens other than Ara h 1. To further address this question, it would be quite interesting to examine the frequency of Ara h 1-reactive T cells in subjects that have been recently diagnosed with peanut allergy. It would also be fruitful to investigate T cells specific for other peanut allergens such as Ara h 2. Indeed data from a recent study in France

suggested that sensitization to Ara h 2 is more predictive for peanut allergy diagnosis than sensitization to Ara h 1⁽¹³⁾. As sensitization to Ara h 1 is high in the North American population, monitoring both Ara h 1- and Ara h 2-reactive T cells may be essential for examining cellular immune responses toward peanut in peanut-allergic subjects in North America.

In addition to measuring frequencies, the surface phenotypes of Ara h 1-specific T cells in peanut allergic subjects were examined in this study. While the scope of this study did not allow us to correlate cell phenotype with any clinical characteristics, such as severity of peanut allergy, patterns were evident across our subject population as a whole. As expected, Ara h 1-specific cells expressed the memory T cell marker CD45RO, and did not express CD45RA, a naïve marker. The majority also expressed CCR4, a Th2-associated cell trafficking marker. In contrast, only a minority of these cells expressed CRTh2, which is thought to be the most definitive Th2 marker. This result was clearly not because of a technical failure to label CRTh2 in our assay, because pollen specific T cells from multiple peanut allergic subjects were shown to express CCR4 and CRTh2. Interestingly, a higher percentage Ara h 1-reactive T cells expressed CD25 than did the total PBMC in the same subject. The CD25 expression of Ara h 1-reactive T cells occurred in the absence of peanut exposure and is probably correlated to their Th2 phenotype⁽²⁰⁾. Despite the fact that CD25 expression is also linked to suppressive T cell function⁽²⁷⁾, some caution is warranted in concluding that allergen-specific CD4⁺CD25⁺ T cells have regulatory function, as these cells were shown to produce robust levels of cytokine (as discussed below). Expression of the skin homing marker CLA was not statistically different on Ara h 1-reactive T cells as compared to total CD4⁺ T cells. In contrast, examination of the gut homing marker $\alpha\beta 7$ on Ara h 1-reactive T cells indicated that these cells express significantly less $\beta 7$ integrin than do the total CD4⁺ T cells. This outcome was unexpected, because Ara h 1-reactive T cells would be likely to first encounter peanut allergens in the gastrointestinal system, which is accessed by $\alpha\beta 7$ expression. One possible explanation is that $\beta 7^{+}$ Ara h 1-reactive T cells are sequestered within the gut and secondary lymphoid tissue and do not circulate in the peripheral blood.

Cytokine profiles of peanut-reactive T cells were also examined in this study. As might be expected, the vast majority of Ara h 1-specific T cell lines and clones examined produced Th2 cytokines such as IL-4 and IL-5. These included cell lines that produced IL-4 only and cell lines that produced IL-4 and IL-5 simultaneously. Analysis of culture supernatants indicated that IL-5 producing cell lines could also produce IL-13. In addition, cells that produced IL-10 were also observed. One powerful application of tetramers is the antigen specific analysis of the co-production of cytokines on the single cell level. We found IL-4 and IL-10 co-producers, as well as cells that produced only IL-10. It is likely the IL-10 produced by IL-4/IL-10 co-producing cells functions in an autocrine fashion to down-regulate IL-4 production. Ara h 1-reactive T cells that produced IL-17 were also observed. Among the IL-17 producers, a very small percentage produced both IL-4 and IL-17. The presence of IL-4 and IL-17 co-producers in asthmatic subjects has been previously reported by Cosmi et al⁽²⁸⁾. Oseroff et al⁽²⁹⁾ also reported the presence of Timothy grass-specific IL-17 producing cells in subjects with Timothy grass allergy. Both groups indicated IL-17 producing cells represented a small subset of the cells in their studied cell population. These prior findings concur with the current data, in that only one of the sixteen Ara h 1-reactive lines examined produced IL-17. Somewhat surprisingly, Ara h 1-specific IFN- γ producing cells were also observed. IFN- γ has been implicated in the down-regulation of Th2 responses and also in the recruitment of Th2 cells to sites of inflammation^(30;31). The current study did not address the various roles of these different T helper subsets. Nevertheless, these current findings demonstrate that Ara h 1-specific CD4⁺ T cells have multifunctional capacities in peanut allergic subjects. Among these, Th2-type CD4⁺ T cells

are likely to act as the dominant players, with Th1, Th17 and regulatory cells of identical antigen specificity also playing important roles.

As previously mentioned, most of the Ara h 1-specific cells examined did not express CRTh2, the most definitive Th2 marker. However, CCR4 staining results and cytokine secretion profiles clearly indicated that these cells are functionally Th2-like. The fact that the subjects from this study have avoided contact with peanut allergens for a long period of time may have resulted in a lack of CRTh2 expression by Ara h 1-reactive T cells. This would suggest that occasional antigen stimulation is essential for the expression of CRTh2 by T cells. This notion is supported by our observation that 75% (6/8) of Ara h 1 specific T cell clones expressed various levels of CRTh2 after activation and subsequent resting (data not shown). However, it is also possible that these Ara h 1-specific cells could be CRTh2-negative for a functional reason and may play a role in the pathophysiology of allergy distinct from CRTh2-positive Th2 cells.

This study highlights the use of tetramer reagents to detect and characterize Ara h 1-reactive CD4⁺ T cells. Its findings demonstrate the feasibility of using Ara h 1-specific tetramers as a specific biomarker for monitoring peanut-specific CD4⁺ T cells in various settings. Although the frequency of Ara h 1-reactive CD4⁺ T cells is relatively low in peanut allergic subjects, there was a significant difference in the frequency of Ara h 1 CD4⁺ reactive T cells in allergic subjects compared with non-allergic subjects. More importantly, tetramer reagents can be used to monitor the surface phenotype of peanut reactive T cells. As such, immune monitoring with tetramers can provide unambiguous data regarding the function, frequency and phenotype of allergenic reactive T cells. These reagents, used independently or in conjunction with other assays, may open up new approaches to monitor changes in the phenotype and frequency of food allergen-reactive CD4⁺ T cells during immunotherapy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

APC	allophycocyanin
FITC	fluorescein isothiocyanate
Aln g	<i>Alnus glutinosa</i>
Ara h	<i>Arachis hypogaea</i>
HLA	human histocompatibility leukocyte antigen
PBMC	peripheral blood mononuclear cell
PE	phycoerythrin
PerCP	peridinin chlorophyll protein
PHA	phytohemagglutinin

Phl p *Phleum pratense*

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Key Messages

- Peanut-specific T cells could be detected in peanut-allergic subjects using class II tetramers.
- Most of these were Th2 cells, but cells that secreted IFN- γ , IL-10 and IL-17 were also detected.

Figure 1.

Frequencies of Ara h 1 epitope-reactive T cells. A. Frequencies of Ara h 1₃₂₁₋₃₄₀-specific T cells in a DR1101 allergic subject and a DR1101 non-atopic subject. The frequencies of Ara h 1-specific T cells per million CD4+ T cells are as indicated. B. Frequencies of Ara h 1 epitope-reactive T cells in 11 peanut allergic subjects, 6 non-atopic subjects and 5 peanut non-allergic atopic subjects. Each data point represents the frequency of T cells specific for a single epitope in Ara h 1. A Student *t* test was used in the statistical analysis. * $P < 0.05$.

Figure 2.

Phenotype of Ara h 1-reactive T cells. A. PBMC of a DR1101 subject with peanut allergy were stained with PE-labeled DR1101/Ara h 1₃₂₁₋₃₄₀ tetramers and a panel of antibodies. B. Comparison of *ex vivo* phenotypes of Ara h 1-reactive and total CD4+ T cells for multiple peanut-allergic subjects. Ara h 1-reactive cells are denoted by circles, total CD4+ T cells by triangles. A Student *t* test was used in the statistical analysis. * *P* < 0.05.

Figure 3.

Intracellular cytokine staining of Ara h 1-reactive cell lines. First row: an IL-4 and IL-5 producing DR1101- restricted Ara h 1₁₆₉₋₁₈₈-stimulated cell line. Second row: an IL-4 and IL-10 producing DR0401-restricted Ara h 1₃₂₉₋₃₄₈-stimulated cell line. Third row: an IFN- γ producing DR1401-restricted Ara h 1₃₂₁₋₃₄₀-stimulated cell line. Fourth row: an IL-4 and IL-17 producing DR0404-restricted Ara h 1₃₂₉₋₃₄₈-stimulated cell line. Fifth row: an IL-4 and IL-5 producing DR0401-restricted Ara h 1₅₇₇₋₅₉₆-specific T cell clone. Each percentage shown indicates the percentage of cytokine producing cells from the tetramer positive population.

Figure 4.

Dual cytokine analysis of Ara h 1-reactive T cell lines. PBMC were stimulated with Ara h 1 peptide for 2 weeks and stained with tetramers. Tetramer positive cells were gated and cytokine profiles were analyzed by ICS staining. A. IL-4 and IL-5 producing DR1101-restricted Ara h 1₁₆₉₋₁₈₈-reactive T cells from Allergic Subject #5 (Table I). B. IL-4 and IL-10 producing DR0401-restricted Ara h 1₃₂₉₋₃₄₈-reactive T cells from Allergic Subject #1. C. IL-4 and IL-17 producing DR0404-restricted Ara h 1₃₂₉₋₃₄₈-reactive T cells from Allergic Subject #2. Each percentage shown indicates the percentage of cytokine producing cells from the tetramer positive population. Data shown is representative of at least three independent experiments.

Table 1

HLA and allergic status of recruited subjects

Subject#	Sex	Age	HLA	Asthma	Peanut IgE Immunocap
Peanut allergic individuals					
1	F	60	DR0401/DR1501-B5	No	3
2	M	34	DR0404/DR0803	No	5
3	M	23	DR0401/DR1302	No	4*
4	F	24	DR0701/DR1401	Yes	3
5	F	49	DR1101/DR1302	Yes	4
6	M	36	DR0101/DR1501-B5	Yes	3^
7	M	22	DR0101/DR0701	No	3^
8	M	26	DR0101/DR1301	Yes	2
9	M	20	DR0301/DR0701	Yes	5
10	M	35	DR0901/DR1101	No	4
11	F	19	DR0701/DR1502-B5	Yes	6
12	F	33	DR0301/DR0701	Yes	5
Non-atopic subjects					
13	F	30	DR0101/DR0103	No	0
14	F	30	DR0101/-	No	0
15	M	39	DR0401/DR1101	No	0
16	F	30	DR0401/DR1401	No	0
17	F	28	DR1501-B5/-	No	0
18	M	29	DR0401/DR1501-B5	No	0
Peanut non-allergic atopic subjects					
19	M	38	DR0401/DR1401	No	0 [#]
20	M	Unknown	DR0401/DR1501-B5	No	0 ^{^A}
21	M	50	DR1101/DR1501-B5	Yes	0 ^{^A}
22	F	26	DR0901/DR1502-B5	No	0 [^]
23	F	33	DR0301/DR0401	No	0*

* Subjects also had history of Timothy grass allergy and positive IgE Immunocap for Timothy grass

Subjects also had history of Alder pollen allergy and positive IgE Immunocap for Alder

Subject also had history of Cat allergy and positive IgE Immunocap for Cat

TABLE II

Ara h 1 CD4+ T cell epitopes

	Ara h 1	Amino Acid Sequence
DRB1*0101	Ara h 1 (201–220)	QRSRQFQNLQNHRIVQIEAK
	Ara h 1 (233–252)	DNILVIQQGQATVTVANGNN
	Ara h 1 (505–524)	KEGDVFIMPAAHPVAINASS
DRB1*0301	Ara h 1 (409–428)	NNFGKLFVVKPDKKNPQLQD
DRB1*0401	Ara h 1 (201–220)	QRSRQFQNLQNHRIVQIEAK
	Ara h 1 (329–348)	FNEIRRVLLEENAGGEQEER
	Ara h 1 (505–524)	KEGDVFIMPAAHPVAINASS
	Ara h 1 (577–596)	QKESHFVSARPSQSQSPSS
DRB1*0404	Ara h 1 (329–348)	FNEIRRVLLEENAGGEQEER
	Ara h 1 (449–468)	NSKAMVIVVVNKG TGNLELV
DRB1*1101	Ara h 1 (169–188)	TSRNNPFYFPSRRFSTRYGN
	Ara h 1 (321–340)	LEAAFNAEFNEIRRVLLEEN
	Ara h 1 (457–476)	VVNKG TGNLELVAVRKEQQQ
	Ara h 1 (465–484)	LELVAVRKEQQQGRREEEE
DRB1*1401	Ara h 1 (321–340)	LEAAFNAEFNEIRRVLLEEN
	Ara h 1 (329–348)	FNEIRRVLLEENAGGEQEER
DRB1*1502	Ara h 1 (201–220)	QRSRQFQNLQNHRIVQIEAK
DRB5	Ara h 1 (209–228)	LQNHRIVQIEAKPNTLVLPK
	Ara h 1 (369–388)	SKEHVEELTKHAKSVSKKGS
	Ara h 1 (489–508)	EEEGSNREVRRTARLKEGD

Table III

Cytokine profiles of Ara h 1 clones and lines

HLA restriction	epitope	Cell line/Clone	IFN- γ	IL-4	IL-5	IL-10	IL-17
DR0101	Ara h 1 ₂₀₁₋₂₂₀	Line 1	-	++	-/+	-	-
DR0101	Ara h 1 ₂₀₁₋₂₂₀	Line 2	-	++	ND	ND	-
DR0401	Ara h 1 ₂₀₁₋₂₂₀	Clone 9	-	++	++	-	-
DR0401	Ara h 1 ₃₂₉₋₃₄₈	Clone 3	-/+	++	++	-	-
DR0401	Ara h 1 ₃₂₉₋₃₄₈	Clone 7	-	++	-	-	-
DR0401	Ara h 1 ₅₇₇₋₅₉₆	Clone 19	-	++	++	-	-
DR0401	Ara h 1 ₃₂₉₋₃₄₈	Line 1	+	ND	ND	++	-
DR0401	Ara h 1 ₃₂₉₋₃₄₈	Line 2	+	++	-	+	-
DR0401	Ara h 1 ₃₂₉₋₃₄₈	Line 3	-/+	++	-	++	-
DR0404	Ara h 1 ₃₂₉₋₃₄₈	Line 1	-	++	-	-	-
DR0404	Ara h 1 ₃₂₉₋₃₄₈	Line 2	-/+	++	-/+	-	++
DR1101	Ara h 1 ₁₆₉₋₁₈₈	Line 1	-	++	++	-	-
DR1101	Ara h 1 ₁₆₉₋₁₈₈	Line 2	-/+	++	++	-	-
DR1101	Ara h 1 ₁₆₉₋₁₈₈	Line 3	+	++	++	+	-
DR1101	Ara h 1 ₃₂₁₋₃₄₀	Line 1	-/+	++	++	-/+	-
DR1401	Ara h 1 ₃₂₁₋₃₄₀	Line 1	++	+	-/+	-	-

< 5% positive in ICS staining is -

6% to 10% is -/+

11% to 30% is +

Greater than 30% is ++