

Peanut T-cell epitope discovery: Ara h 1

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Background: Identification of potential T-cell epitopes in the peanut major allergens is essential for development of peptide-based immunotherapy. Traditional methods of T-cell epitope discovery use overlapping short peptides spanning the full length of the protein in T-cell proliferation assays. Because large proteins, such as Ara h 1, require a large number of peptides, this limits screening to a small number of allergic subject-derived T-cell lines.

Objective: We sought to identify candidate peptides of Ara h 1 that display promiscuous binding to MHC class II and induce TH2 cytokine production by T cells.

Methods: *In silico* MHC class II binding prediction was performed with NetMHCIIpan 2.0 (peptide length, 15; 1-mer offset) and the most abundant class II alleles in the North American population and with an *in vitro* MHC class II peptide reporter assay performed in parallel, which used synthetic 15-mer peptides offset by 5 mer spanning the protein. High-resolution MHC class II typing and a T-cell proliferation assay using preselected peptides were performed with PBMCs from 98 subjects with peanut allergy and 14 healthy control subjects. IL-4, IL-13, IL-5, IFN- γ , and TNF- α levels were measured in culture supernatants.

Results: Thirty-six Ara h 1 peptides were identified by using *in silico* predictions and MHC class II binding assays. In combination with T-cell proliferation and cytokines secreted in

T-cell assays, we have identified 4 vaccine candidate Ara h 1 peptides.

Conclusions: Preselection of peptides by using *in silico* and *in vitro* approaches in combination with conventional methods appears to be an effective strategy for identifying peanut T-cell peptide vaccine candidates. (J Allergy Clin Immunol 2016;■■■:■■■-■■■.)

Key words: Ara h 1, peanut, food allergy, peptide epitope

Peanut allergy is the most common food allergy and cause of food-induced anaphylaxis among school-aged children in the United States,^{1,2} with low spontaneous resolution.³ Several approaches have been attempted to treat peanut allergy, including subcutaneous immunotherapy,⁴ oral immunotherapy (OIT),^{5,6} and sublingual immunotherapy.⁷ These are associated with high rates of adverse reactions or low efficacy.⁸ Compared with crude peanut extract (CPE),⁹ peptide-based immunotherapy using T cell epitope-containing peptides (too short to effectively cross-link allergen-specific IgE on mast cells and basophils) might provide efficacious desensitization without significant reactions. This has been achieved in allergic subjects with Fel d 1 for cat allergy,¹⁰ phospholipase A₂ for vespid allergy¹¹ and with ovalbumin (egg)¹² and Ara h 1 in mice with egg and peanut allergy.¹³ Unlike cat allergy, in which a single immunodominant protein is responsible for allergy in the majority of patients, several allergenic proteins have been identified in peanut, including Ara h 1, Ara h 2, Ara h 3, Ara h 6, Ara h 8, and Ara h 9. Most patients are sensitized to Ara h 1, Ara h 2, or Ara h 3. More than 90% of subjects with peanut allergy have antibodies against Ara h 1. Ara h 1 is a 614-amino-acid seed storage protein belonging to the vicilin family of proteins. We previously characterized the T-cell epitopes of Ara h 2 using *in vitro* T-cell stimulation assays with multiple overlapping 20-mer peptides that spanned the length of the protein.¹⁴ The length of the amino acid sequence of Ara h 1 is more than twice that of Ara h 2. Therefore utilization of an overlapping peptide approach is expensive and impractical because of the requirement of large volumes of blood for T-cell assays. *In silico* MHC class II epitope prediction (NetMHCIIpan 2.0) for Ara h 2 retrospectively identified a similar set of peptides as likely T-cell epitopes, as predicted by using T-cell stimulation assays.¹⁴ Here we used a similar *in silico* epitope discovery algorithm prospectively and combined it with an *in vitro* HLA-binding assay (Pro-immune REVEAL assay) to preselect peptides. These were then evaluated by T-cell stimulation assays. Cytokine production and stimulation data were combined to determine immunotherapeutic candidates.

METHODS

Subjects

A total of 112 subjects (98 subjects with peanut allergy and 14 healthy control subjects) were recruited for this study from the outpatient clinic at the

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Abbreviations used

CPE: Crude peanut extract
 CV: Coefficient of variation
 kU_A: Kilounits of allergen
 OIT: Oral immunotherapy

Jaffe Food Allergy Institute at the Icahn School of Medicine at Mount Sinai. The eligibility criteria for recruitment included a convincing clinical history of an immediate-type reaction to peanut and a positive skin prick test response, positive serum IgE level, or both to peanut or a peanut-specific IgE level of greater than 15 kilounits of allergen per liter (kU_A/L). Subjects who had omalizumab treatment within the past 12 months and enrolled in any food allergy interventional trial in the past 12 months or current treatment with immunotherapy for any allergen in the build-up phase were excluded from the study. This study was approved by the institutional review board and complied with all guidelines of the institutional Program for the Protection of Human Subjects. Informed consent was obtained from all patients.

T-cell epitope prediction and MHC binding assays

T-cell epitope prediction was performed with the artificial neural network-based alignment method NetMHCIIpan 2.0 (<http://www.cbs.dtu.dk/services/NetMHCIIpan/>), as described previously.¹⁴ Peptide-MHC binding assays were performed by high-throughput ProImmune REVEAL assays (ProImmune, Oxford, United Kingdom). This method identifies epitopes that bind MHC class II based on conformational changes induced in MHC class II molecules on peptide binding. Peptide-bound MHC class II molecules are recognized by using a conformation-specific labeled antibody to provide a binding readout. Binding to MHC molecules was compared with that of 2 known control T-cell epitopes. The REVEAL binding score for each peptide-MHC complex is calculated by comparison with binding of the relevant positive control. Scores of more than 15% of control binding indicated significant or strong binders. A library of 121 peptides (15 mer) staggered by 5 residues was synthesized and assayed in this *in vitro* class II binding assay.

T-cell proliferation assay with selected Ara h 1 peptides

Selected Ara h 1 20-mer peptides offset by 5 amino acids were synthesized by JPT Peptide Technologies GmbH (Berlin, Germany). The peptides were dissolved in sterile PBS (4 µg/20 µL), plated in 96-well U-bottom culture plates, and stored at -80°C until use. Freshly isolated PBMCs were isolated from whole blood by means of Ficoll density centrifugation, and 1×10^6 PBMCs were cultured for 4 days in 96-well U-bottomed plates with CPE, anti-CD3/CD28 beads, or Ara h 2 peptides (final concentration, 4 µg/200 µL) in AIM-V medium. On day 4, 100-µL aliquots of the culture supernatant were removed and stored at -80°C, and 100 µL of fresh AIM-V medium was added to the cells. Cultures were then pulsed with tritiated thymidine (1 µCi) for 48 hours and harvested on glass fiber filters, and incorporated radioactivity was measured in counts per minute by using scintillation spectroscopy. Individual counts per minute for Ara h 1 peptides, CPE, and anti-CD3/CD28 bead culture wells were compared with those of cultures containing medium alone (unstimulated cells) and plated in triplicates. The within-subject variability of the counts per minute measured among these triplicates was calculated and used to estimate the per-individual coefficient of variation (CV). T-cell responses were considered significant if the stimulation index (counts per minute of antigen-stimulated T cells divided by counts per minute of unstimulated T cells) exceeded the estimated maximum CV ($>1 + CV_{\max}$); otherwise, they were regarded as negligible (zero). Peptides inducing positive proliferative responses (stimulation index ≥ 1.5) in at least 25% of the recruited subjects were considered significant. Cytokine analysis (IL-5, IL-13, TNF-α, and IFN-γ) was performed in cell-culture supernatants collected on day 4 by using Luminex technology (Luminex, Austin, Tex), according to the manufacturer's instructions.

TABLE I. Demographic and clinical parameters

Parameter	Value
Age (y)	13.8 ± 4.9
Male sex, no. (%)	66 (67.3)
Race, no. (%)	
White	83 (84.7%)
Black	2 (2.0)
Asian	4 (4.1)
Hispanic	1 (1.0)
Other (specify)	3 (3.1)
Unknown	7 (7.1)
Other food allergies,* no. (%)	
Tree nut	73 (74.5)
Soy	8 (8.2)
Sesame	22 (22.4)
Legumes	20 (20.4)
Other	28 (28.6)
Other allergies, no. (%)	
Allergic rhinitis	72 (73.5)
Asthma	56 (57.1)
Atopic dermatitis	50 (51.0)
Skin prick test response (mm)	
Wheal	11.6 ± 5.7
Flare	27.0 ± 14.8
Specific IgE (kU _A /L)	61.4 ± 38.6

*Per patient report.

HLA class II genotyping

Genomic DNA was isolated from venous blood (QIAamp DNA Mini Kit; Qiagen, Hilden, Germany) and used to perform high-resolution HLA-DRB1* typing (by using Luminex technology; Bio-Synthesis, Lewisville, Tex). Allele (number of copies of the allele) and phenotype (number of subjects who carry the allele) frequencies were calculated in our cohort.

Basophil degranulation assay

In brief, 250 µL of whole blood from subjects with peanut allergy was stimulated for 30 minutes at 37°C with peanut (2 µg/mL), anti-IgE (1 µg/mL), a mixture of selected Ara h 1 peptides (40, 20, or 10 µg/mL), or medium alone. The degranulation reaction was stopped by adding 20 mmol/L EDTA, followed by staining with fluorescent-conjugated mAbs against the following markers: CD63, CD123, CD203c, CD41a, CD3, CD14, CD19, and HLA-DR. The cells were washed, acquired in a BD LSR II Flow Cytometer, and analyzed with FACSDiva software (version 9.7.6; BD Biosciences, San Jose, Calif). Basophils were gated as CD203⁺CD123⁺HLA-DR^{dim/-}CD41a⁻CD3⁻CD14⁻CD19⁻ cells. The frequency of CD63⁺ basophils was assessed as a marker of degranulation and compared with each condition.

RESULTS**Demographics**

A total of 98 subjects (66 male and 32 female) with peanut allergy were recruited (Table I). The average age was 13.8 ± 4.9 years. Eighty-three subjects were white, and 84 (82.3%) had other food allergies. Most patients had coexisting tree nut allergies. Eighty-six (84.3%) patients had other allergic problems, including asthma, allergic rhinitis, and/or atopic dermatitis. The demographics and clinical parameters are summarized in Table I. The subjects were HLA typed, and their HLA frequencies are summarized in Table E1 in this article's Online Repository at www.jacionline.org.

In silico-predicted MHC binding affinity of peptides

The full-length Ara h 1 sequence was analyzed by using the NetMHCIIpan 2.0 server.¹⁵ All possible 15-mer peptides with

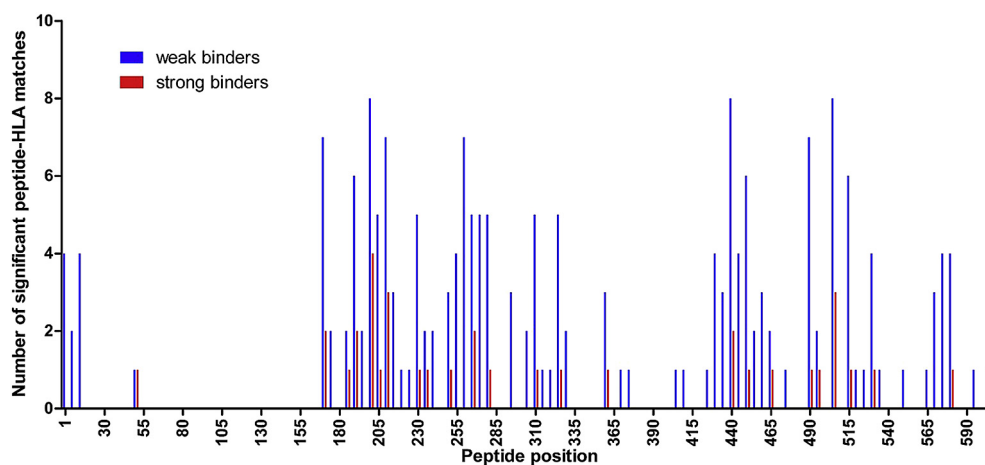


FIG 1. Full-length Ara h 1 was analyzed for HLA-DRB binding affinity for the HLA alleles indicated in Table E2; binding affinity was reported in nanomoles per liter. Thresholds for peptides that were strong (inhibitory concentration of 50%) and weak binders were 50 and 500 nmol/L, respectively. Numbers of HLA types binding with strong or weak affinity at each amino acid start position (*x-axis*) are indicated.

5-mer stagger were analyzed for predicted binding to the most common HLA-DRB1 alleles in our population. The predicted binding strengths were reported. Peptides with predicted affinities of less than 50 nmol/L and less than 500 nmol/L were reported as strong and weak binders, respectively. Fig 1 (see also Table E2 in this article's Online Repository at www.jacionline.org) shows the number of weak and strong binders by start position of the peptides. Most predicted epitopes are concentrated in certain hotspots (amino acid positions 170-230, 270-370, and 430-530).

***In vitro* observed MHC binding affinity of peptides**

The ProImmune REVEAL assay, which uses a reporter system to identify MHC peptide binding, was used to analyze peptide binding of all 15-mer peptides staggered by 5 mer to HLA-DRB1*01:01, DRB1*15:01, DRB1*03:01, DRB1*11:01, DRB1*13:01, DRB1*07:01, DRB1*04:02, DRB1*11:04, DRB1*15:02, and DRB1*15:03. The affinity of peptides with significant binding to these HLA types is displayed in Fig 2. One hundred eighteen peptides from the ProImmune REVEAL assay were compared with *in silico* results from NetMHCIIpan by using a similar set of HLA-DRB alleles. Of these, 32 peptides were predicted to be class II binders by both assays (see Fig E1 in this article's Online Repository at www.jacionline.org). Overall, 22 peptides were predicted to be HLA binders by using the *in silico* analysis but not by using the REVEAL assay, whereas 5 peptides demonstrated MHC binding *in vitro* but were not predicted to bind by using *in silico* analysis. The 22 peptides that were predicted to be HLA binders but did not bind any HLA in the ProImmune assays were observed to have significantly lower mean predicted binding affinities (0.73 ± 0.09) compared with the peptides that were identified by both assays (1.87 ± 0.35 , $P < .05$). We observed that a number of peptides predicted to bind HLA-DRB1*03:01, DRB1*13:01, DRB1*07:01, and DRB1*11:04 using *in silico* analysis did not bind on the ProImmune assay. Combining REVEAL data and *in silico* predictions and solubility analysis, we identified 36 peptides for further analysis (see Table E3 in this article's Online Repository at www.jacionline.org).

Peptide-specific T-cell proliferation *ex vivo*

PBMCs were isolated from subjects with peanut allergy and nonallergic control subjects. Plasma was separated and stored. PBMCs were cultured with peptides, whole peanut protein, anti-CD3 antibody, or medium control. T-cell proliferation was measured based on thymidine incorporation. Cytokine concentrations were measured in supernatants. Proliferation assays from 90 subjects showed appropriate proliferative responses with the control stimuli, such as CPE or anti-CD3/anti-CD28 beads. Fig 3, A, shows the number of peptides to which each subject with peanut allergy or control subject responded. Subjects with peanut allergy responded to significantly greater numbers of peptides (5.678 ± 0.8162 , $n = 90$) compared with nonallergic control subjects (1.43 ± 0.33 , $n = 14$). We then analyzed the number of subjects with peanut allergy who responded to each peptide (Fig 3, B). A minimum of 8 and a maximum of 19 subjects responded to each peptide (mean $\pm$ SD = 13.78 ± 2.8). There was no significant difference in the number of patients responding to each peptide ($P = .92$, χ^2 test). T cells from 8 subjects proliferated in response to whole peanut but not to Ara h 1 peptides. These subjects were HLA diverse.

Cytokine production

To further characterize the recall responses triggered by these peptides, we measured cytokine levels in tissue culture supernatants that showed measurable proliferation to peptides or experimental controls in allergic and nonallergic subjects. We measured IL-4, IL-13, IFN- γ , IL-5, and TNF- α levels (Fig 4). Although proliferative responses to some peptides were associated with greater cytokine production, all peptides were associated with a T_H2 -dominant phenotype characterized by large amounts of IL-13 production. Levels of the inflammatory cytokine TNF- α were also increased in allergic subjects compared with those in nonallergic subjects. On the other hand, cytokine production from proliferating cells in nonallergic control subjects was at least 5 to 100 times lower than seen in allergic subjects for all cytokines.

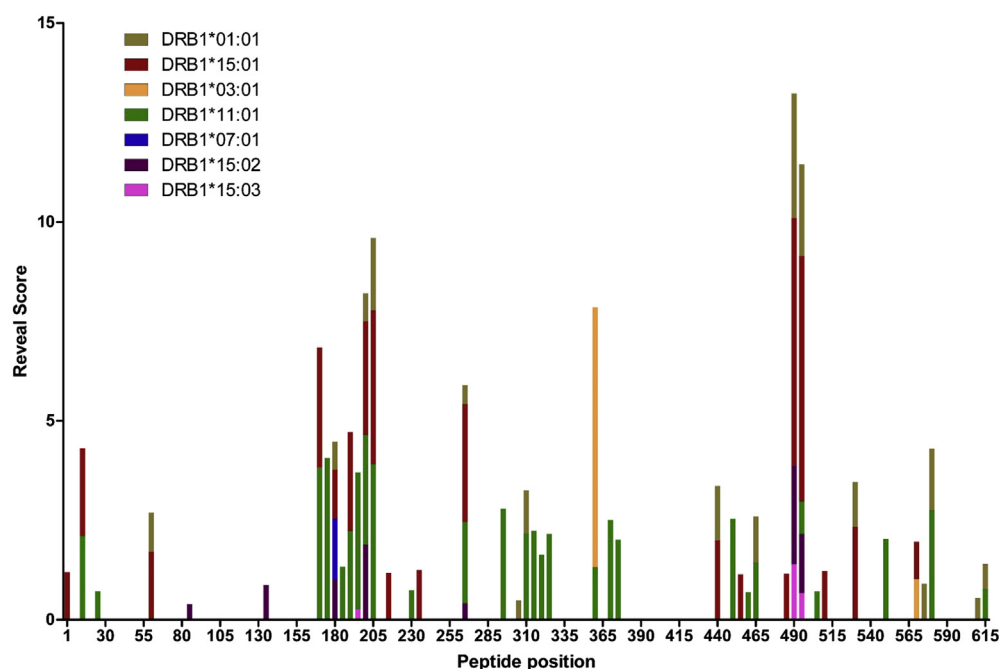


FIG 2. 15-mer Peptides with a 5-mer stagger were evaluated for *in vitro* HLA binding in the ProlImmune REVEAL assay. The REVEAL scores for peptide-HLA combinations with significant binding affinities (>15%) are displayed for each amino acid start position (*x-axis*).

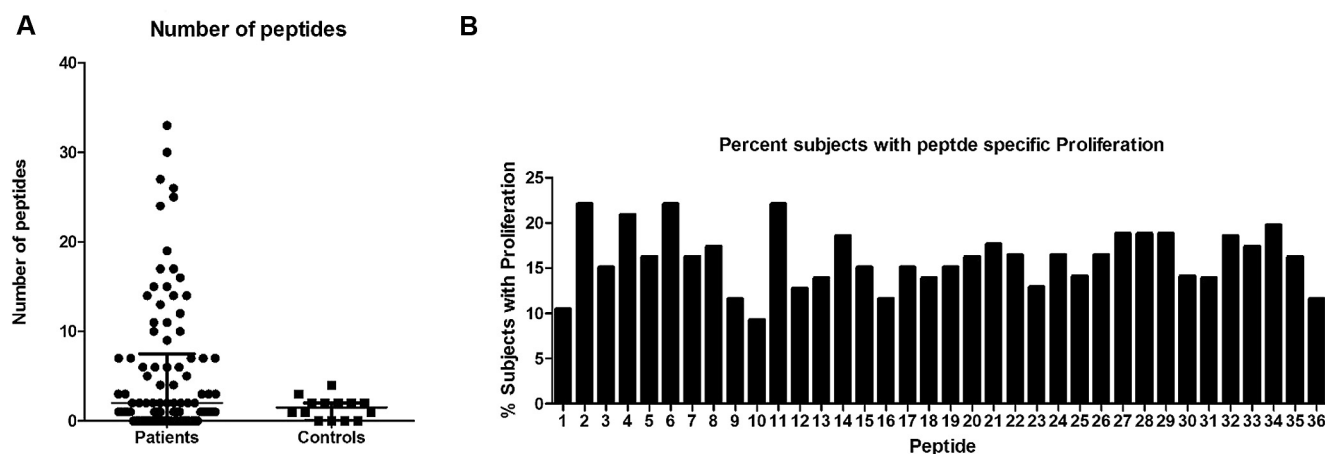


FIG 3. Peptide-specific T-cell proliferation responses. T-cell proliferation was measured in peptide-, crude peanut allergen-, or anti-CD3 bead-stimulated T cells by using a thymidine incorporation assay and reported as the stimulation index. Patient-peptide combinations with a stimulation index of greater than 1.5 were reported as positive. **A**, Number of peptides to which T cells of each subject with peanut allergy or nonallergic subject proliferated. **B**, Number of subjects whose T cells proliferated in response to each peptide (*x-axis*).

Ara h 1-specific IgE and Ara h 1 peptide-induced cytokine production

We measured Ara h 1-specific antibodies in sera after enrollment of study patients. Of the 8 subjects who did not respond to any Ara h 1 peptide but had crude peanut antigen-specific responses, 6 had detectable Ara h 1-specific IgE (Ara h 1-specific IgE ≥ 0.35 kU_A/L, Fig 5). The serum specific IgE concentrations were not different between the subjects who responded (17.09 ± 10.39 kU_A/L) or did not respond to our set of Ara h 1 peptides (42.58 ± 6.658 kU_A/L). Conversely, we noted

that 11 allergic subjects had Ara h 1 peptide-specific T-cell responses but did not produce Ara h 1-specific IgE (Ara h 1-specific IgE <0.35 kU_A/L). We segregated these peanut- but not Ara h 1-sensitized subjects from Ara h 1-sensitized subjects and compared their cytokine responses. Subjects sensitized to peanut but not Ara h 1 responded to significantly fewer Ara h 1 peptides compared with subjects with Ara h 1 allergy (3.6 ± 1.4 [*n* = 8] vs 9.1 ± 1.3 [*n* = 43], *P* < .01), although we observed no difference in IL-13 production (*P* = .50, 2-way ANOVA). Interestingly, those subjects who had

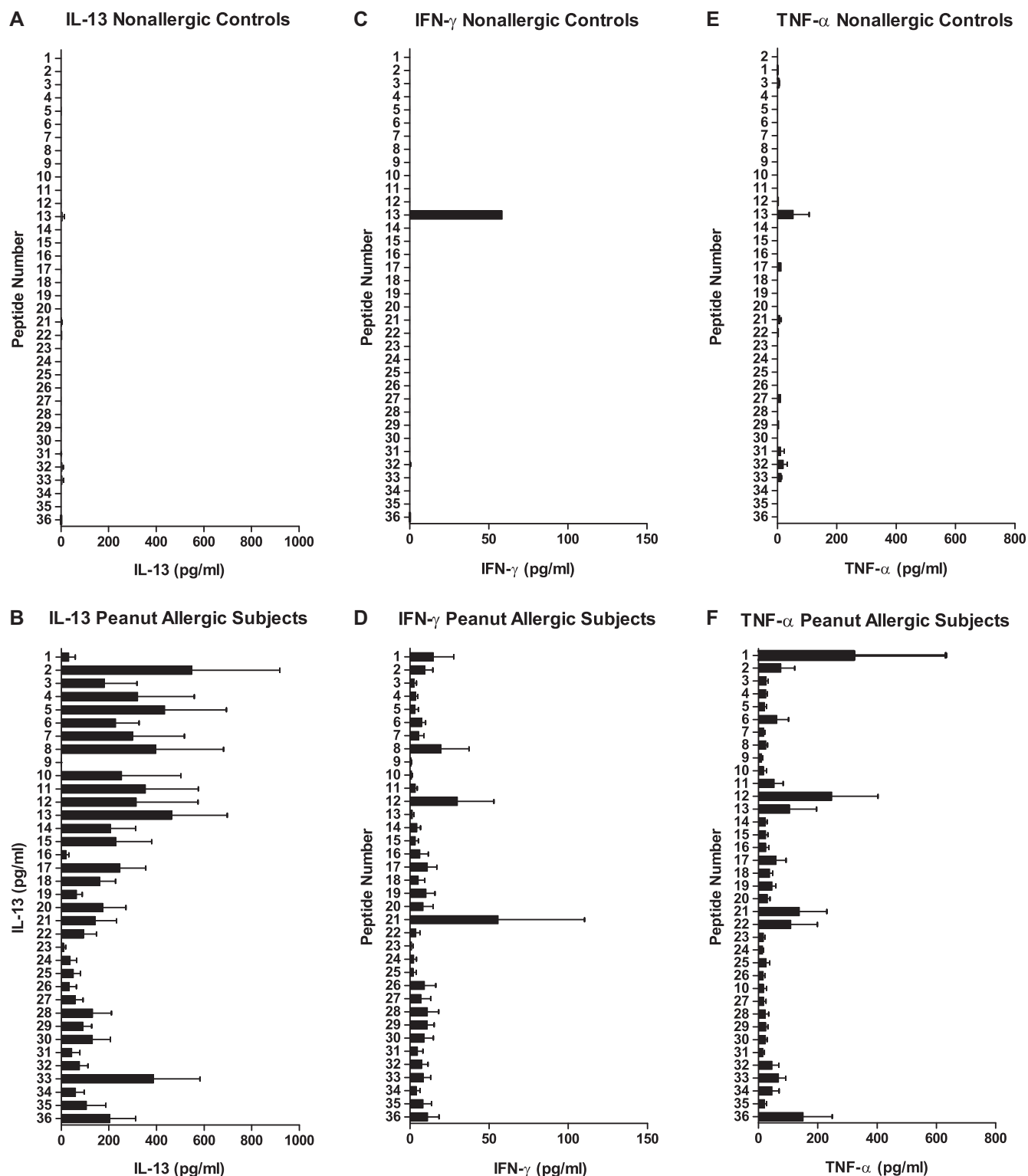


FIG 4. Cytokine production in T-cell stimulation assays. IFN- γ (A and B), IL-13 (C and D), and TNF- α (E and F) levels were measured in supernatants of the peptide-specific T-cell stimulation assays in nonallergic subjects (Fig 4, A, C, and E) and subjects with peanut allergy (Fig 4, B, D, and F). Cytokine levels were measured in wells with a stimulation index of greater than 1.5. Bars represent mean cytokine levels in proliferating wells, and whiskers represent SEMs.

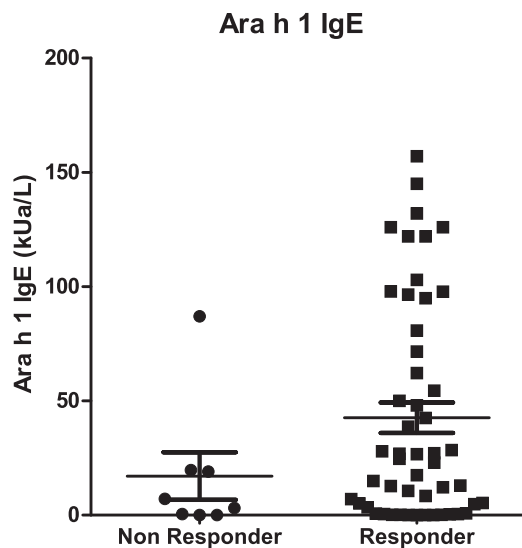


FIG 5. Ara h 1 IgE levels in peptide responders and nonresponders. Ara h 1–specific IgE levels were measured in subjects whose T cells proliferated in response to any of the peptides (responders, $n = 51$) and those whose T cells did not proliferate with Ara h 1 peptides but did proliferate in response to crude peanut antigen (nonresponders, $n = 8$).

Ara h 1–specific T cells (as evidenced by their proliferative responses) but did not have Ara h 1–specific IgE had significantly higher IFN- γ production than the Ara h 1–sensitized subjects (Fig 6).

Safety of Ara h 1 peptides

All candidate peptide sequences were 15 amino acids long. Although this is not considered long enough to cross-link cell membrane-bound IgE, it is critical to demonstrate that these peptides or macromolecular complexes that might form in solution do not result in mast cell degranulation. To this end, we obtained whole blood from 4 subjects with peanut allergy and performed basophil degranulation assays in the presence of as select mix of 7 peptides that induced the highest levels of T-cell proliferation (Fig 7). Basophils from all 4 subjects showed a robust degranulation with crude peanut extract or anti-IgE (positive controls). However, as expected, Ara h 1 peptides did not induce degranulation at all 3 different concentrations tested.

DISCUSSION

The standard of care for managing food allergy remains strict avoidance of the culprit food and education regarding the recognition and emergency management of allergic reactions. OIT, sublingual immunotherapy, and epicutaneous immunotherapy are in various phases of development. However, no approved therapies for peanut allergy (or other food allergies) are yet available.^{16–18} Moreover, studies have consistently demonstrated that the adverse reaction rate to OIT is higher than would be expected with avoidance alone and that benefit is at best limited to temporary desensitization and not sustained unresponsiveness for the majority of patients.

New-generation peptide-based immunotherapy targeting T cells with short peptides that eliminate IgE epitopes has

demonstrated efficacy without significant adverse side effects.¹⁹ Peptide-based immunotherapy has shown efficacy in patients with Hymenoptera venom, cat, dust mite, and ragweed allergies.^{11,19–21} These therapies rely on a well-established role of T cells in food and environmental allergies.²²

Ara h 1 epitopes have been mapped in 2 previous studies.^{23,24} These studies have used 2 different approaches for evaluating candidate epitopes: tetramer-guided epitope mapping and carboxyfluorescein succinimidyl ester–based proliferation assays by using T-cell clones generated from allergic subjects. These methods have high specificity but low sensitivity.²⁵ Thus, ultimately, both methods require external validation with patient samples.²⁵ These studies assayed peptide pools in 12 and 22 patients, respectively. The obvious strengths in our report are that we used a rapid identification method for generating candidate peptides combining 2 “patient-free” methodologies, *in silico* prediction and *in vitro* HLA-binding assays, and enrolled a larger cohort of subjects with peanut allergy. In a previous study we mapped the sequential T-cell epitopes of Ara h 2 using T-cell stimulation assays. We validated *in silico* discovery of T-cell peptides by comparison with experimental results, demonstrating a high degree of reproducibility between the 2 methods.¹⁴ As evidenced by PBMC proliferative responses, none of the peptides tested in our study had a significantly lower responder frequency. The prescreening methodology only screened HLA-DRB alleles. Additional prescreening might provide a broader pool. Of 600 possible overlapping 15-mer peptides, only 36 were assayed in *ex vivo* stimulation assays, and yet all but 6 samples from subjects with Ara h 1 allergy demonstrated T-cell proliferation in response to this pool of preselected peptides. Ara h 1 antibody in these subjects might arise from T-cell help rendered by T cells that recognize epitopes not covered in our pool or secondary to bystander help from T cells with other specificities.²⁶

T-cell responses in allergic subjects are characterized by the predominance of T_H2 cytokine secretion, whereas T-cell responses in nonallergic subjects are characterized by the dominance of T_H1 cytokines.²⁷ Our data corroborate these findings, with nonallergic subjects appearing anergic with little cytokine production. It is worth noting that subjects with peanut allergy who had undetectable Ara h 1–specific IgE had a T_H1 predominance in their cytokine responses. Allergen-induced T_H2 cytokine levels have been shown to be decreased after peptide immunotherapy for cat allergy in human subjects²⁸ and for egg allergy in mice.¹² Unlike regulatory T cell–mediated suppression, of which there is little evidence, studies have consistently demonstrated cytokine deviation associated with clinically effective peptide-based allergen immunotherapy.^{28,29} We envisage that changes in peptide-specific cytokine responses could be used as a biomarker during peptide-based immunotherapy. Component-specific differences in T-cell responses are a novel finding and could potentially be a marker of immunotherapy outcomes.

In conclusion, using a multipronged approach, we have identified the immunodominant T-cell epitope sequences of Ara h 1. These peptides do not induce basophil degranulation by IgE crosslinking and are likely to be safer than other therapies, being highly unlikely to provoke anaphylaxis. Use of these sequences along with other peanut T-cell epitopes in a peptide-based immunotherapy regimen will provide a novel treatment strategy for peanut allergy.

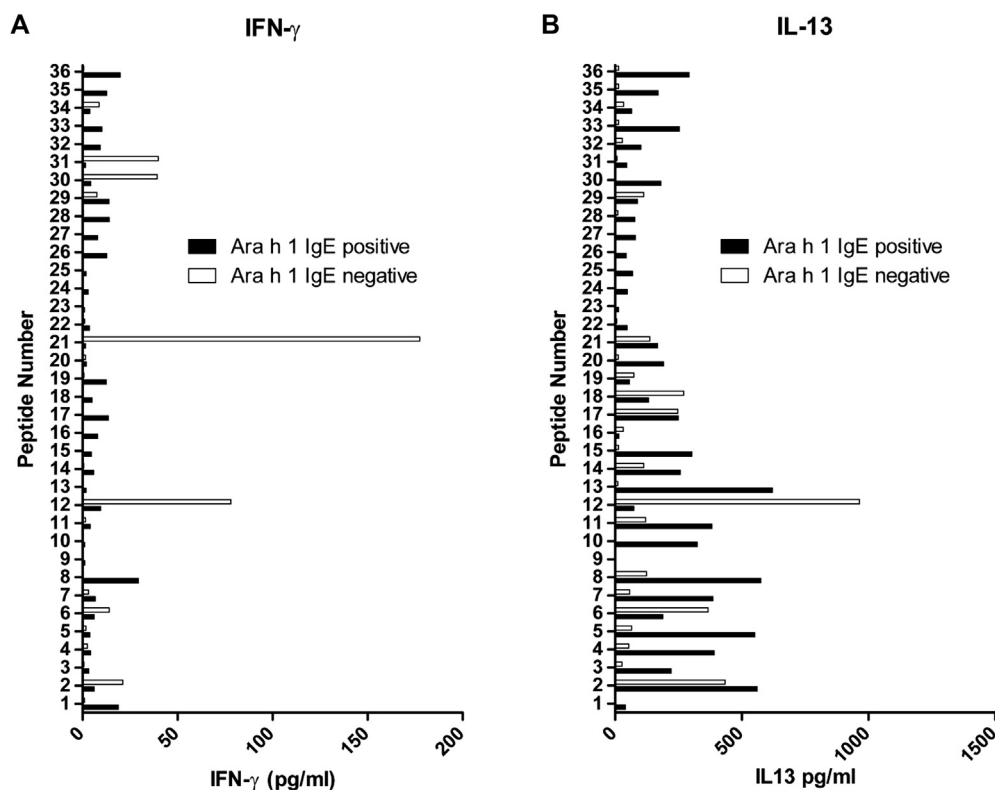


FIG 6. IFN- γ and IL-13 levels in proliferation assays in subjects with and without Ara h 1 allergy. IFN- γ (A) and IL-13 (B) levels were measured in supernatants of T-cell proliferation assays in subjects with peanut allergy who had positive (black bars) or negative (white bars) results for Ara h 1-specific IgE.

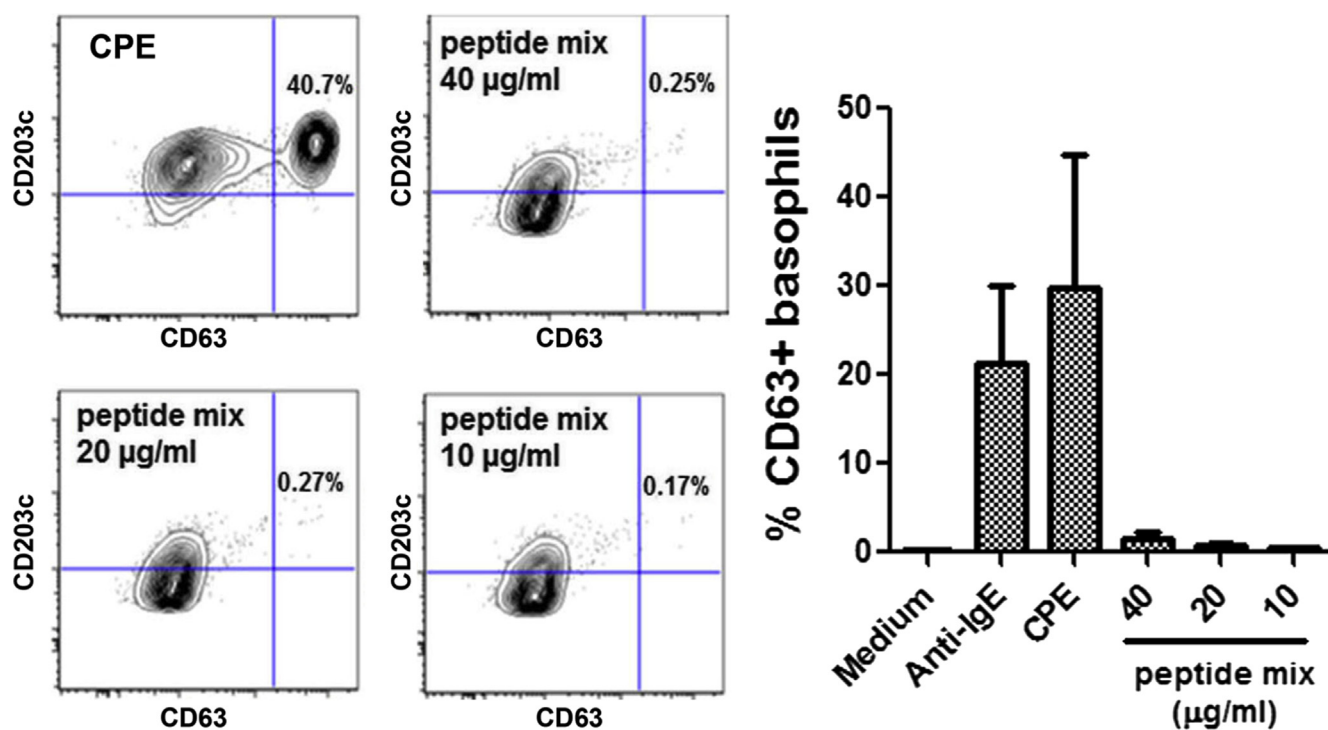


FIG 7. Candidate peptide mix does not cause basophil cell degranulation. Donor basophils from subjects with peanut allergy were cultured with medium, anti-IgE, crude peanut antigen, or peptide mix of candidate peptides at 3 concentrations (40, 20, and 10 μ g/mL each). Representative flow cytometric plots and percentages of CD63⁺ basophils are shown (mean $\pm$ SEM). FITC, Fluorescein isothiocyanate; PE, phycoerythrin; PNE, peanut extract.

Key messages

- We have identified immunodominant T-cell epitope sequences of the major peanut allergen Ara h 1.
- *In silico* Ara h 1 T-cell epitope prediction, *in vitro* MHC-binding assays, T-cell proliferation assays, and cytokine release data were combined to determine immunotherapeutic candidates.

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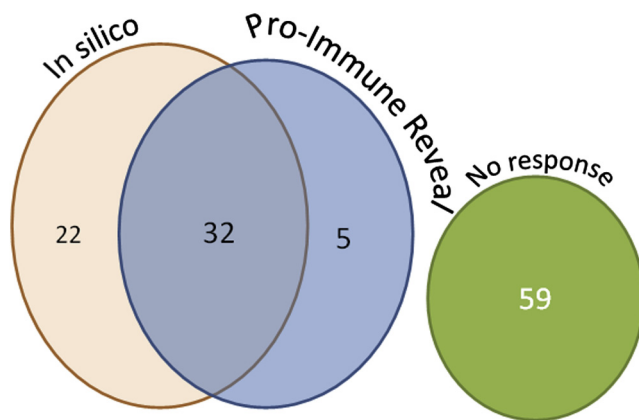


FIG E1. All Ara h 1-derived 15-mer peptides staggered by 5 mer were assayed for HLA binding in the *in silico* prediction assay NetMHCIIpan or the ProImmune REVEAL assay. The number of peptides with significant predicted or measured responses in these assays, respectively, are represented in a Venn diagram.

TABLE E1. Measured HLA frequencies

HLA type	No. (frequency)*	HLA type	No. (frequency)*	HLA type	No. (frequency)*
HLA-DPB*04:01	24 (30.0%)	HLA-DQB*03:01	22 (25.3%)	HLA-DRB*04:01	9 (10.3%)
HLA-DPB*02:01	16 (20.0%)	HLA-DQB*05:01	9 (10.3%)	HLA-DRB*15:01	9 (10.3%)
HLA-DPB*04:02	11 (13.8%)	HLA-DQB*06:02	9 (10.3%)	HLA-DRB*13:02	8 (9.2%)
HLA-DPB*03:01	8 (10.0%)	HLA-DQB*06:03	7 (8.0%)	HLA-DRB*13:01	7 (8.0%)
HLA-DPB*01:01	6 (7.5%)	HLA-DQB*02:01	5 (5.7%)	HLA-DRB*03:01	6 (6.9%)
HLA-DPB*05:01	3 (3.8%)	HLA-DQB*03:02	5 (5.7%)	HLA-DRB*01:02	5 (5.7%)
HLA-DPB*33:01	3 (3.8%)	HLA-DQB*02:02	4 (4.6%)	HLA-DRB*11:01	5 (5.7%)
HLA-DPB*14:01	2 (2.5%)	HLA-DQB*04:02	4 (4.6%)	HLA-DRB*01:01	4 (4.6%)
HLA-DPB*06:01	1 (1.3%)	HLA-DQB*05:03	4 (4.6%)	HLA-DRB*07:01	4 (4.6%)
HLA-DPB*10:01	1 (1.3%)	HLA-DQB*06:04	4 (4.6%)	HLA-DRB*11:04	3 (3.4%)
HLA-DPB*15:01	1 (1.3%)	HLA-DQB*06:09	4 (4.6%)	HLA-DRB*13:03	3 (3.4%)
HLA-DPB*17:01	1 (1.3%)	HLA-DQB*03:03	3 (3.4%)	HLA-DRB*14:01	3 (3.4%)
HLA-DPB*23:01	1 (1.3%)	HLA-DQB*06:01	3 (3.4%)	HLA-DRB*15:02	3 (3.4%)
HLA-DPB*38:01	1 (1.3%)	HLA-DQB*03:04	1 (1.1%)	HLA-DRB*04:03	2 (2.3%)
HLA-DPB*70:01	1 (1.3%)	HLA-DQB*04:01	1 (1.1%)	HLA-DRB*04:04	2 (2.3%)
		HLA-DQB*05:02	1 (1.1%)	HLA-DRB*04:07	2 (2.3%)
		HLA-DQB*10:01	1 (1.1%)	HLA-DRB*08:04	2 (2.3%)
				HLA-DRB*09:01	2 (2.3%)
				HLA-DRB*12:01	2 (2.3%)
				HLA-DRB*04:02	1 (1.1%)
				HLA-DRB*08:02	1 (1.1%)
				HLA-DRB*13:05	1 (1.1%)
				HLA-DRB*13:09	1 (1.1%)
				HLA-DRB*14:02	1 (1.1%)
				HLA-DRB*16:01	1 (1.1%)

*HLA alleles on either chromosome were counted, and the frequency at either locus was determined.

TABLE E2. HLA types assayed

HLA-DRB alleles
DRB1*0101
DRB1*0301
DRB1*0402
DRB1*0701
DRB1*1101
DRB1*1104
DRB1*1301
DRB1*1501
DRB1*1502
DRB1*1503

TABLE E3. Ara h 1 peptide solubility (number of neutral peptides = 4 [peptides 2, 15, 20, and 32])

	Amino acids	Sequence	Charge	Attribute
1	16-30	VLASVSATHAKSSPY	2	Basic
2	61-75	RCTKLEYDPRCVYDP	0	Neutral
3	171-185	RNNPFYFPSRRFSTR	4	Basic
4	191-205	GRIRVLQRFDQRSRQ	4	Basic
5	196-210	LQRFDQRSRQFQNLQ	2	Basic
6	201-215	QRSRQFQNLQNHRIV	4	Basic
7	206-220	FQNLQNHRIVQIEAK	2	Basic
8	212-226	HRIVQIEAKPNTLV	2	Basic
9	233-247	DNILVIQQGQATVTV	−1	Acidic
10	243+257	ATVTVANGNNRKSFN	2	Basic
11	253-267	RKSFNLDGEGHALRIP	2	Basic
12	271-285	ISYILNRHDNQNLRV	2	Basic
13	280-294	NQNLRVAKISMPVNT	2	Basic
14	296-310	GQFEDFFPASSRDQS	−2	Acidic
15	311-325	SYLQGFSRNTLEAAF	0	Neutral
16	321-335	LEAAFNAAFNEIRRV	−1	Acidic
17	326-340	NAEFNEIRRVLLEEN	−2	Acidic
18	361-375	NEGVIVKVSKEHVEE	−1	Acidic
19	371-385	EHVEELTKHAKSVSK	2	Basic
20	405-419	PDLNNFGKLFVVKP	0	Neutral
21	416-430	EVKPKKKNPQLQDL	−1	Acidic
22	425-439	QLQDLDMMLTCVEIK	−2	Acidic
23	431-445	MMLTCVEIKEGALML	−1	Acidic
24	441-455	GALMLPHFNSKAMVI	2	Basic
25	451-460	KAMVIVVVNKG TGNL	2	Basic
26	466-480	ELVAVRKEQQQRGR	3	Basic
27	491-505	EGSNREVRRTARLK	3	Basic
28	496-510	EVRRYTARLKEGDVF	1	Basic
29	506-520	EGDVFIMPAAHPVAI	−1	Acidic
30	516-530	HPVAINASSELHLLG	1	Basic
31	531-545	FGINAENNHRIFLAG	1	Basic
32	536-550	ENNHRIFLAGDKDNV	0	Neutral
33	551-565	IDQIEKQAKDLAFPG	−1	Acidic
34	569-583	QVEKLIKQKESHFV	2	Basic
35	581-595	HFVSARPQSQSQSPS	2	Basic
36	612-626	QGGKGPLLSILKAFN	2	Basic