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EXPERIMENTAL ALLERGY AND IMMUNOLOGY

Kinetics, cross-reactivity, and specificity of Bet v 1-specific IgG4 antibodies induced by immunotherapy with birch pollen

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Keywords

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Abstract

Background: IgE antibodies specific for the major birch pollen allergen frequently cross-react with Bet v 1 homologous food proteins, for example Cor a 1 in hazelnut and Mal d 1 in apple. Specific immunotherapy with birch pollen (BP-SIT) induces IgG4 antibodies that inhibit IgE binding to Bet v 1. However, information on cross-reactivity of BP-SIT-induced Bet v 1-specific IgG4 antibodies with food allergens is limited. In this study, we investigated the kinetics of production, cross-reactivity, and IgE-blocking activity of Bet v 1-specific IgG4 antibodies emerging during conventional BP-SIT and whether IgG4-epitopes overlapped with IgE epitopes. **Methods:** IgE and IgG4 levels specific for Bet v 1, Mal d 1, and Cor a 1 were determined in 42 birch pollen-allergic patients before and during BP-SIT. Inhibition of IgE binding was studied by IgE-facilitated antigen-binding assays and basophil activation tests. Furthermore, inhibition of IgE-mediated activation of food allergen-reactive Bet v 1-specific T-cell lines was assessed. Competitive immunoscreening of phage-displayed peptides was applied to select mimotopes recognized by IgE and IgG4 antibodies, respectively. The resulting mimotopes were mapped on the surface of the 3D structure of the allergens using a computer-based algorithm. **Results:** BP-SIT significantly increased Bet v 1- and food allergen-reactive IgG4 antibodies. In parallel, allergen-specific IgE levels decreased significantly. Sera containing food allergen-reactive IgG4 antibodies inhibited IgE binding, basophil activation, and IgE-mediated food allergen-induced T-cell proliferation. Predicted IgE and IgG4 epitopes on all allergens showed high overlap. **Conclusion:** Our results indicate that BP-SIT may induce Bet v 1-specific IgG4 antibodies that cross-react with related food allergens and inhibit IgE binding by epitope competition.

Allergen-specific immunotherapy (SIT) is an effective treatment of IgE-mediated hypersensitivity to birch pollen (1). Successful BP-SIT has been associated with the induction of

Bet v 1-specific IgG4 Abs that are capable of inhibiting IgE binding to the major birch pollen allergen (2). Consequently, IgE-mediated activation of effector cells and Bet v 1-specific T lymphocytes is reduced (3–5).

Bet v 1, the major birch pollen allergen, has been well characterized regarding its structure (6) and B- and T-cell epitopes (7–10). Bet v 1 shares IgE epitopes with structurally related food proteins, for example Cor a 1 in hazelnut and Mal d 1 in apple (9, 11). These Bet v 1 homologs show

Abbreviations

A, amino acid; Ab, antibody; BAT, basophil activation test; BP, birch pollen; FAB, facilitated antigen binding; OAS, oral allergy syndrome; SIT, allergen-specific immunotherapy; TCL, T-cell line.

67.3% and 55.7% amino acid (aa) sequence identity with Bet v 1, respectively, and a very similar three-dimensional structure (12). So far, only very few studies have assessed whether BP-SIT-induced IgG4 Abs recognize Bet v 1-related food allergens and exert blocking activity (13–15). This tempted us to study the kinetics of production, cross-reactivity, and IgE-blocking capacity of BP-SIT-induced Bet v 1-specific IgG4 Abs. We quantified IgE and IgG4 levels specific for Bet v 1, Cor a 1, and Mal d 1 in sera from 42 patients before and at different time points during therapy. IgE-blocking activity was assessed by IgE-facilitated allergen-binding (FAB) assays (16), basophil activation tests (17), and IgE-facilitated antigen presentation to allergen-specific T cells (5).

We found Bet v 1-specific IgG4 Abs that cross-reacted with Cor a 1 and Mal d 1 and exerted IgE-blocking activity. Currently, it is not known whether this blocking is due to competition of IgG4 for IgE epitopes or sterical hindrance after binding of IgG4 to sites different from IgE epitopes. To address this aspect, we performed competitive immunoscreening of phage-displayed peptides (9) and predicted IgE and IgG4 epitopes on Bet v 1, Mal d 1, and Cor a 1 using the algorithm EpiSearch (18).

Materials and methods

Allergens

Recombinant Bet v 1.0101 (termed 'Bet v 1') and recombinant Mal d 1.0801 (termed 'Mal d 1') were purchased from Biomay, Vienna, Austria. Recombinant Cor a 1.0401 (Cor a 1) was kindly provided by S. Scheurer, Paul-Ehrlich Institut, Langen, Germany.

Patients

Birch pollen-allergic patients (age range 10–57 years, median 35 years) who received their first SIT were included. P1-P9 received ALK-depot SQ108 (ALK-ABELLO, Hørsholm, Denmark) (3), and blood samples were collected as follows: months 0, 1, 3, 12, 24, and 36 out of the birch pollen season; months 6, 18, and 30 during the birch pollen season. P10-P42 received ALK-ALUTARD SQ197 (ALK-ABELLO), and all blood samples were collected out of the birch pollen season. Both vaccines contain Al(OH)₃ as adjuvant. The cumulative dose of Bet v 1 was around 150 µg for 1 year and around 500 µg for 3 years of SIT.

Assessment of allergen-specific antibodies

Allergen-specific IgE and IgG4 Abs were quantified by ImmunoCAP (Thermo Fisher Scientific, Uppsala, Sweden).

IgE-FAB assay

For all tests, the same indicator serum was used. Indicator serum (20 µl) containing >100 kU/l of birch pollen-specific IgE and test sera (15 µl) or an equal volume of medium was pre-incubated with titrated concentrations (1, 0.1,

0.01 µg/ml) of allergens (5 µl) for 1 h at 37°C. Then, EBV-transformed B cells ($1 \times 10^5/5 \mu\text{l}$) were added and incubated for 1 h at 4°C. After washing and saturation with 20% human AB serum for 20 min at 4°C, cells were stained with FITC-labeled anti-human IgE (KPL, Gaithersburg, MD, USA). After washing, IgE-positive B cells were detected by flow cytometry using a FACS Canto and Diva software (BD Biosciences, San Jose, CA, USA). All assays were performed in duplicate, and 5000 viable cells per sample were acquired.

Basophil activation tests (BAT)

Heparinized blood from untreated birch pollen-allergic patients was incubated for 30 min at 37°C with different concentrations of Bet v 1 or Cor a 1 (100, 10, 1, 0.1, 0.01 ng/ml) in RPMI supplemented with human IL-3 (5 ng/ml; PeproTech, Rocky Hill, NJ, USA). Medium alone served as negative and anti-human IgE (1 µg/ml; Nordic Immunological Laboratories, Maidenhead, UK) as positive control. After adding PBS containing 20 mM EDTA, cells were stained with CD63-PE, CD123-FITC (both from Biolegend, San Diego, CA, USA), CD203c-APC, CD45-PerCP (both from Miltenyi Biotec, Bergisch Gladbach, Germany), or the respective isotype controls (all from BD Biosciences). After lysis of erythrocytes, the percentage of CD63⁺ cells in the CD45⁺ CD123⁺ population was assessed by flow cytometry. All analyses were performed in duplicate. For inhibition experiments, allergens (1 ng/ml) were pre-incubated with sera from SIT-treated patients for 1 h at 37°C prior to the addition to whole blood.

Bet v 1-specific T-cell lines (TCL)

Bet v 1-specific TCL were generated from non-SIT-treated birch pollen-allergic individuals by stimulating PBMC (1.5×10^6) with Bet v 1 (10 µg/ml) as described in the study by Mutschlechner et al. (19). Bet v 1-specific TCL cross-reactive with Mal d 1 and/or Cor a 1 were expanded to cell numbers necessary for inhibition experiments. After incubation of allergens (each at 0.01 µg/ml in 5 µl) with indicator serum (20 µl) plus test sera (20 µl) or an equal volume of medium, EBV-transformed B cells ($5 \times 10^4/5 \mu\text{l}$) were added. After washing twice, cells were incubated for 2 h at 37°C, irradiated (60 Gy), and added to Bet v 1-specific TCL (5×10^4 cells/well). All experiments were performed in duplicate. After 48 h, proliferation was assessed by uptake of ³[H] thymidine (0.5 µCi/well) and expressed as mean counts per minute (cpm).

Competitive immunoscreening of phage-displayed peptides

Competitive immunoscreening of a Ph.D.-12 Phage Display Peptide Library (New England BioLabs, Beverly, MA, USA) was performed as described with slight modifications (9). Briefly, 2.5 mg of Dynabead M-280 tosylactivated beads (Invitrogen Dynal AS, Oslo, Norway) was coupled with 50 µg mouse anti-human IgE or IgG4 Abs (Southern Biotech, Birmingham, AL, USA) at 37°C overnight, blocked with 0.5% BSA in PBS at RT for 2 h, and incubated with patients' sera (500 µl) at 4°C overnight. After extensive washing, beads were

resuspended in 200 μ l of PBS containing 0.1% BSA. Then, 100 μ l was used for the first round of selection, while the remaining 100 μ l was kept for the second round of selection.

First round of panning

Beads (100 μ l) were incubated with 2^{11} phages for 3 h at RT. After extensive washing, bound phages were amplified by direct infection of ER2738 *Escherichia coli* cells at 37°C for 4.5 h. Phages were purified by PEG precipitation and tittered.

Second round of panning

Beads were incubated with 2^{11} phages selected from the first round. After extensive washing, beads were resuspended in 300 μ l of PBS containing 0.1% BSA. For specific elution, beads (100 μ l) were incubated with 400 μ l of PBS, 0.1% BSA containing 25 μ g of either Bet v 1, Mal d 1, or Cor a 1 for 1 h at RT. Eluted phages were amplified, purified, and tittered. Single plaques were picked and amplified, and amino acid (aa) sequences of the selected phage-displayed peptides were determined by DNA sequencing using M13 phage-specific primers.

Mapping of peptides onto the allergen surface

Epitopes were predicted using the default settings for 12-mer peptides of 'EpiSearch' (<http://curie.utmb.edu/episearch.html>) (18). This algorithm matches the sequences of antibody-binding peptides with surface-exposed aa in patches of similar physicochemical nature as the input peptides on the 3D structure of the allergen. Only peptides with highest matching scores >1.0 were considered as putative epitopes. The following three-dimensional experimentally solved or modeled structures of the allergens were used for epitope prediction: Bet v 1 (PDB code: 1BV1) (6), Mal d 1, and Cor a 1 (20).

Defining overlapping IgE and IgG4 epitopes

Available crystal structures of antibody–antigen interactions suggest that a typical epitope covers ~ 600 – 900 Å² on the surface of an antigen (21, 22). We calculated the solvent-accessible surface areas (ASAs) of predicted IgE and IgG4 epitopes using GETAREA (<http://curie.utmb.edu/getarea.html>) (23).

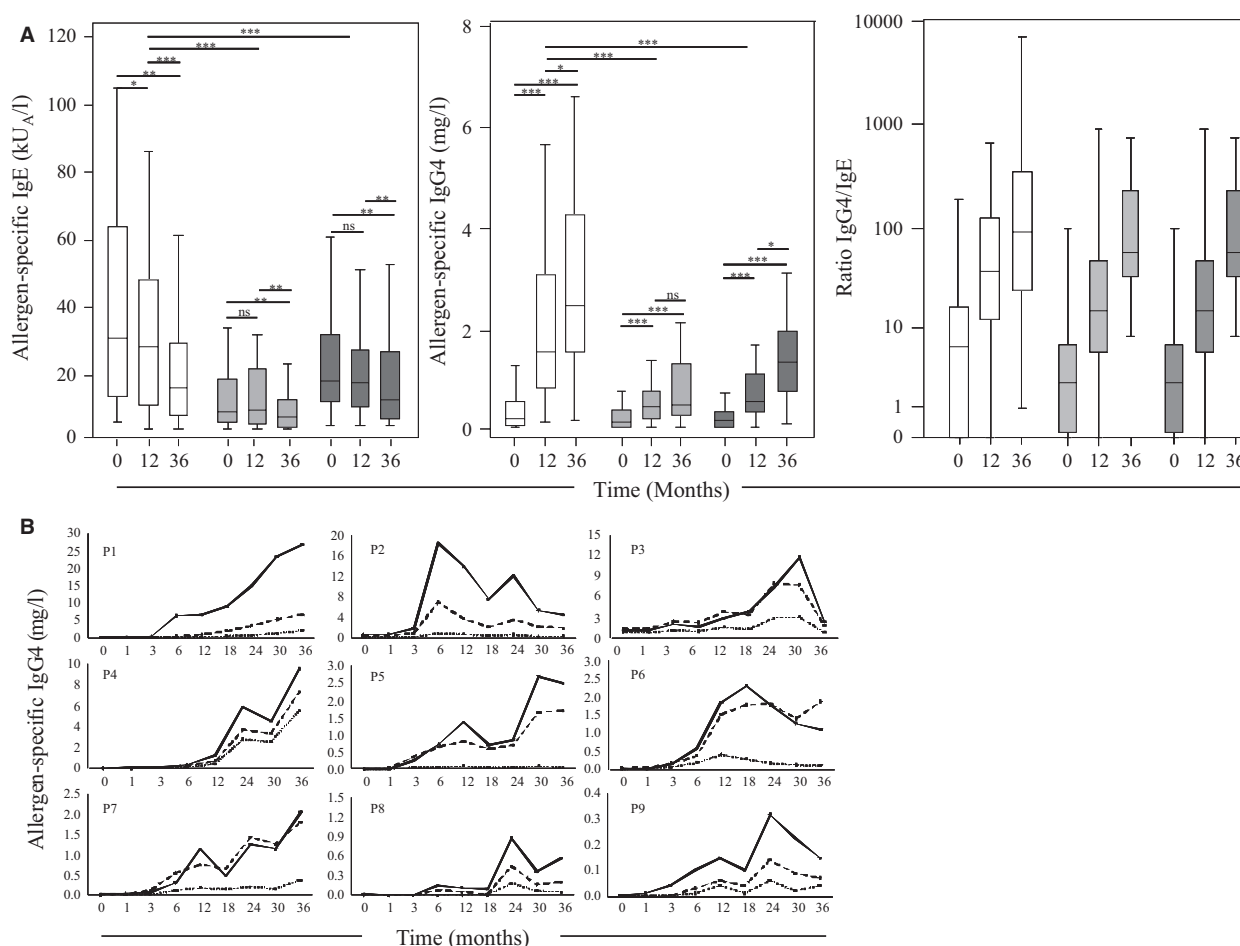


Figure 1 Allergen-specific IgE and IgG4 levels during the course of BP-SIT. (A) Ab levels and IgG4/IgE ratios specific for Bet v 1 (white box plots), Mal d 1 (light gray), and Cor a 1 (dark gray) in 42 sera before (0) and after 12 and 36 months of BP-SIT, respectively.

Wilcoxon signed-rank test, * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$. (B) Time kinetics of IgG4 specific for Bet v 1 (full line), Mal d 1 (dotted line), and Cor a 1 (dashed line) in patients P1–P9.

The ASAs of IgE and IgG4 epitopes were compared. Two epitopes were considered as distinct, if there was $>600 \text{ \AA}^2$ of contiguous area outside the intersection of the two predicted surface patches. Representative examples of overlapping and distinct IgE/IgG4 epitopes are depicted in Fig. 4.

Results

Allergen-specific IgE and IgG4 responses to BP-SIT

In sera from 42 birch pollen-allergic patients collected before, at 12 and 36 months of BP-SIT, Bet v 1-, Mal d 1-, and Cor a 1-specific IgE and IgG4 levels were quantified by ImmunoCAP (Fig. 1). Bet v 1-specific IgE levels continuously decreased during the course of BP-SIT (Fig. 1A, m0/m12, $P = 0.034$; m0/m36, $P = 0.001$; and m12/m36, $P < 0.001$).

Mal d 1- and Cor a 1-reactive IgE levels were significantly reduced at 36 months of BP-SIT (Mal d 1: m0/m36, $P = 0.004$, and m12/m36, $P = 0.001$; Cor a 1: m0/m36, $P = 0.005$, and m12/m36, $P = 0.001$). At each time point, Bet v 1-specific IgE levels were significantly higher than food allergen-reactive IgE levels (m0: Bet v 1/Mal d 1, $P < 0.001$; Bet v 1/Cor a 1, $P < 0.001$; m12: Bet v 1/Mal d 1, $P < 0.001$; Bet v 1/Cor a 1, $P < 0.001$; and m36: Bet v 1/Mal d 1, $P < 0.001$; Bet v 1/Cor a 1, $P = 0.002$). During BP-SIT, a continuous and significant increase in allergen-specific IgG4 levels occurred (Fig. 1A, Bet v 1: m0/m12, $P < 0.001$; m0/m36, $P < 0.001$; and m12/m36, $P = 0.016$; Mal d 1: m0/m12, $P < 0.001$; m0/m36, $P < 0.001$; m12/m36, $P = 0.252$; Cor a 1: m0/m12, $P < 0.001$; m0/m36, $P < 0.001$; and m12/m36, $P = 0.014$). After 12 and 36 months, respectively, Bet v 1-specific IgG4 levels were significantly higher than food allergen-

Table 1 Allergen-specific IgG4 responses before (0) and after 36 months of BP-SIT in patients sensitized to Bet v 1, Mal d 1, and Cor a 1

Patient no	IgE (kU/l)						IgG4 (mg/l)					
	Bet v 1		Mal d 1		Cor a 1		Bet v 1		Mal d 1		Cor a 1	
	0	36	0	36	0	36	0	36	0	36	0	36
P1	21.96	58.82	3.72	9.14	10.06	24.00	0.28	26.80	0.09	2.07	0.06	6.69
P2	59.51	27.74	30.20	15.72	28.40	15.92	0.55	5.29	0.07	0.20	0.33	2.14
P3	71.82	49.25	19.40	10.44	83.60	61.00	1.22	2.41	0.90	0.89	1.42	1.86
P4	23.87	0.35	0.74	0.35	0.88	0.35	0.01	9.46	0.02	5.53	0.03	7.22
P5	20.86	23.87	9.08	10.78	20.40	23.20	0.00	2.49	0.00	0.04	0.01	1.68
P6	9.51	4.75	1.46	1.20	4.12	2.98	0.03	1.10	0.02	0.11	0.04	1.88
P7	1.78	2.12	1.64	0.86	2.54	1.46	0.02	2.05	0.00	0.37	0.02	1.80
P8	14.20	11.94	4.00	4.04	11.66	10.58	0.00	0.56	0.00	0.05	0.00	0.20
P9	69.77	73.87	22.60	19.76	57.00	56.60	0.00	0.15	0.00	0.04	0.00	0.07
P10	13.72	8.04	4.00	3.62	9.50	10.00	0.62	6.50	0.34	3.20	0.48	4.82
P11	4.04	2.34	1.12	1.14	1.86	1.82	0.09	3.42	0.04	1.33	0.05	3.04
P12	30.00	42.00	12.42	22.80	20.40	32.20	0.16	2.42	0.06	0.97	0.08	1.03
P13	59.20	24.80	20.20	8.10	29.80	12.94	0.39	8.75	0.71	0.36	0.69	1.65
P14	51.40	13.28	5.74	1.10	22.80	4.92	0.03	1.92	0.00	0.45	0.03	1.12
P15	6.73	7.06	4.86	2.20	10.38	4.80	0.53	4.20	0.36	1.20	0.52	3.62
P16	39.60	0.35	20.40	4.98	28.40	8.06	0.19	3.36	0.33	0.70	0.41	1.06
P17	64.00	53.00	8.60	5.86	34.00	24.00	0.58	1.63	0.18	1.35	0.25	0.57
P18	18.92	5.06	2.30	0.84	8.60	2.68	0.22	2.33	0.00	0.17	0.00	0.93
P19	60.00	20.20	22.00	4.08	36.80	8.92	0.02	0.64	0.13	0.42	0.01	0.60
P20	96.60	0.70	5.62	8.66	4.20	23.60	0.07	4.54	0.00	0.29	0.00	0.72
P21	34.60	17.64	11.90	3.76	24.40	10.68	0.25	3.15	0.19	0.28	0.26	1.43
P22	41.20	19.20	15.12	10.50	28.80	17.70	5.24	14.20	2.94	7.20	6.49	14.60
P23	63.20	25.80	10.60	5.56	22.60	9.02	0.14	4.06	0.37	1.20	0.12	1.06
P24	37.40	16.00	9.54	3.44	11.26	5.08	0.59	1.27	0.72	0.38	0.32	0.48
P25	14.02	7.02	3.08	1.62	10.80	4.66	0.05	2.34	0.00	0.12	0.04	0.87
P26	22.60	5.32	5.26	0.90	17.18	2.82	0.07	0.31	0.03	1.27	0.06	0.23
P27	50.60	34.88	9.63	4.94	42.62	23.80	0.24	1.01	0.12	0.84	0.06	0.71
P28	72.40	35.60	13.60	11.70	58.62	29.00	0.34	1.96	0.26	3.76	0.42	1.34
P29	24.20	18.74	9.80	7.04	14.94	12.40	0.01	0.12	0.00	0.35	0.00	0.03
P30	116.80	96.60	25.60	20.80	60.20	46.20	0.13	0.45	0.31	0.96	0.02	0.05
P31	23.40	11.70	3.32	2.40	11.00	6.18	1.14	2.24	1.43	3.30	1.47	2.50
P32	63.60	89.20	25.20	38.60	61.60	88.00	1.09	1.94	1.02	3.58	0.80	1.16
P33	72.00	39.32	20.40	13.36	46.20	25.00	0.33	0.57	0.40	0.90	0.13	0.16
P34	31.60	17.78	7.88	4.12	22.60	11.80	0.13	0.22	0.15	0.63	0.12	0.15
P35	25.40	12.14	3.24	0.84	11.60	6.60	0.03	2.20	0.01	1.17	0.02	0.16

Gray boxes highlight patients who show no increase in IgG4 Ab.

reactive IgG4 levels (m12: Bet v 1/Mal d 1, $P = 0.001$; Bet v 1/Cor a 1, $P < 0.001$; and m36: Bet v 1/Mal d 1, $P < 0.001$; Bet v 1/Cor a 1, $P < 0.001$).

Similar production kinetics of Bet v 1 and food allergen-reactive IgG4 Abs during birch pollen SIT

We monitored the course of allergen-specific IgG4 levels in sera from nine birch pollen-allergic individuals collected before and at months 1, 3, 6, 12, 24, 30, and 36 of BP-SIT (P1-P9 in Table 1, obtained from the clinical trials performed by Mobs et al. (2, 24). In general, Bet v 1-specific IgG4 Abs started to rise after 3–6 months of BP-SIT (Fig. 1B). In seven of nine patients, food allergen-reactive IgG4 levels increased at the same time point, whereas two individuals (P1 and P9) showed a delayed rise of food allergen-reactive IgG4 levels. Of note, only one of nine individuals (P4) showed a significant increase in both Mal d 1- and Cor a 1-reactive IgG4 Abs. The remaining eight individuals developed remarkable levels of Cor a 1-reactive IgG4 Abs and rather weak levels of Mal d 1-reactive IgG4 Abs, although all individuals showed IgE reactivity to both food allergens.

Birch pollen SIT does not induce food allergen-reactive IgG4 Abs in all food-sensitized patients

To substantiate the divergent food allergen-reactive IgG4 responses observed in the nine patients shown in Fig. 1B, allergen-specific IgG4 levels before and at 36 months of BP-SIT were compared in additional 26 patients sensitized to

both Mal d 1 and Cor a 1 (Table 1). A difference of >0.05 mg/l, which was the standard deviation of the assay determined by fivefold repeated analyses of three sera, was considered as positive. Accordingly, 6 of 35 (17%) patients showed no increase in Mal d 1-reactive IgG4, and 4 of 35 (11%) did not develop Cor a 1-reactive IgG4. Some individuals developed Mal d 1-reactive Abs, but no Cor a 1-reactive Abs and *vice versa*. Thus, not all patients who showed IgE cross-reactivity with Mal d 1 and Cor a 1 developed cross-reactive IgG4 Abs during BP-SIT.

Post-birch pollen SIT sera containing food allergen-reactive IgG4 Abs show blocking activity

Next, the blocking activity of sera with ample volumes at each time point (before and at months 1, 3, 6, 12, 24, 30, and 36 of BP-SIT) from four patients who had developed Cor a 1-reactive IgG4 was tested in FAB assays. In addition, the sera were compared for their capacity to inhibit Bet v 1- and Cor a 1-induced basophil activation by evaluating CD63 expression. The presence of post-BP-SIT sera containing allergen-specific IgG4 clearly reduced allergen IgE binding to B cells (Fig. 2A) and basophil activation by both Bet v 1 and Cor a 1 (Fig. 2B). The production kinetics of allergen-specific IgG4 are shown in Fig. 2C. Sera containing Bet v 1-IgG4 and no Cor a 1-IgG4 Abs served as controls and inhibited Bet v 1-IgE complex formation, but not Cor a 1-IgE complex formation (data not shown). To elucidate whether food allergen-reactive IgG4 Abs showed equivalent blocking activity for Bet v 1 and Cor a 1, sera from eight additional patients randomly selected from

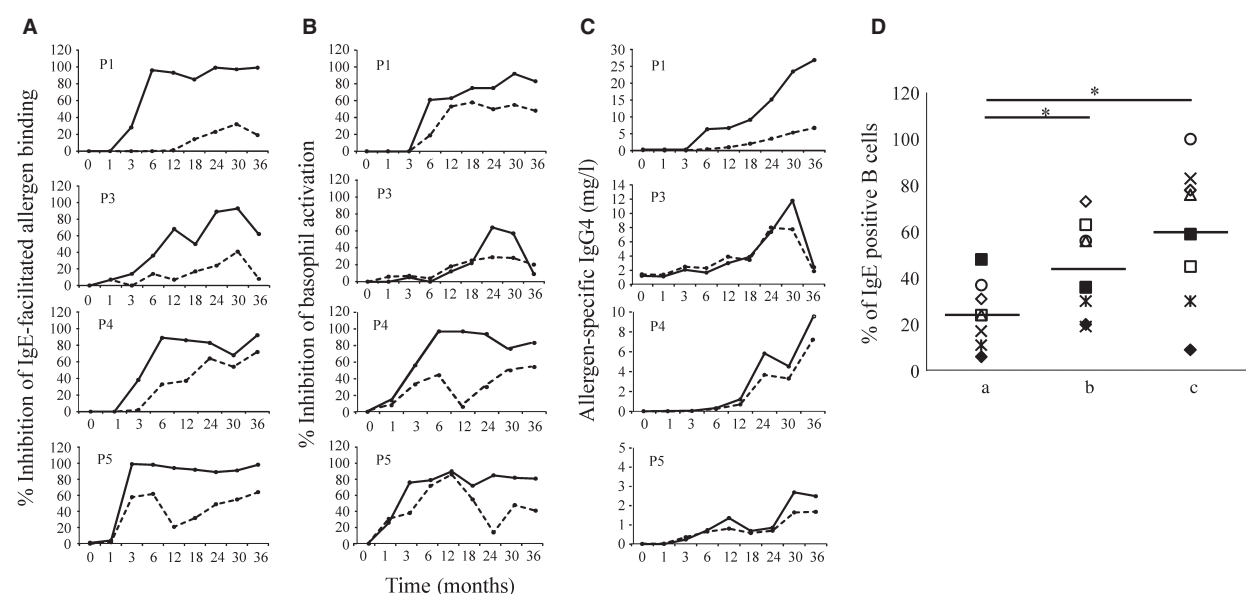


Figure 2 IgE-blocking activity of post-BP-SIT sera. Bet v 1 (full line) and Cor a 1 (dashed line) were pre-incubated with sera from four patients obtained before (0) and during BP-SIT. Pre-BP-SIT sera were normalized to 100%. The percentage of inhibition of (A) basophil activation and (B) IgE allergen binding to B cells is shown. (C) IgG4 levels specific for Bet v 1 (full line) and Cor a 1 (dashed line) in

the respective sera at indicated time points. (D) FAB assays of eight patients who developed Bet v 1- and Cor a 1-reactive IgG4. Sera containing higher levels of Bet v 1- (a) than Cor a 1-reactive (b) IgG4 levels show significantly stronger inhibition activity which is reduced after adjusting Bet v 1- to Cor a 1-reactive IgG4 levels (c). Horizontal lines indicate median values. Wilcoxon signed-rank test, $*P < 0.05$.

(P10-P17 (Table 1) were tested in FAB assays. All sera contained lower levels of Cor a 1- than Bet v 1- specific IgG4 (Table 1). Accordingly, the reduction in Cor a 1-IgE immune complex binding to B cells was significantly lower than inhibition of Bet v 1-IgE complex binding ($P = 0.025$, Fig. 2D). However, after adjusting Bet v 1- and Cor a 1-specific IgG4 levels, a similar inhibitory activity was observed for both allergens (Fig. 2D).

Post-birch pollen SIT sera containing food allergen-reactive IgG4 Abs inhibit IgE-mediated T-cell activation

Bet v 1-specific TCL cross-reactive with the food allergens were stimulated with either Bet v 1, Mal d 1, or Cor a 1 and pre-incubated with sera containing high levels of allergen-specific IgE in the presence of pre- and post-BP-SIT sera from four patients. Sera from three patients contained Bet v 1- and food allergen-reactive IgG4 Abs, and the fourth serum contained Bet v 1-reactive IgG4 Abs, but not food allergen-reactive IgG4 Abs (Fig. 3). Sera inhibited IgE-mediated

proliferation of allergen-specific T-cell lines in response to Bet v 1 and its food homologs (Fig. 3).

Predicted IgE and IgG4 epitopes show a high degree of overlap

For each serum and allergen, individual sets of phage-displayed 12-mer peptides were competitively eluted from IgE- or IgG4-loaded beads with the recombinant allergens, respectively (Table 2). Several peptides were detected multiple times with the same serum and allergen or with different allergens. Moreover, identical peptides were selected with sera from different patients. These findings point to the cross-reactivity of Bet v 1, Cor a 1, and Mal d 1. Of note, nine identical mimotopes bound to both IgE- and IgG4-loaded beads, suggesting that IgE and IgG4 may recognize the same epitopes. All peptides were mapped on the surface of the eluting allergen, and conformational epitopes were predicted by EpiSearch (18) and checked for overlap by GETAREA as described in Materials and Methods (Fig. 4). Table S1 shows all mimotope sequences and aa residues of predicted IgE- and IgG4-epitopes on Bet v 1, Mal d 1 and Cor a 1 for five patients. Table 2 summarizes the total numbers of these IgE- and IgG4-epitopes. For each individual and allergen, the overlap of predicted IgG4 with IgE epitopes ranged from 33 to 100% (mean for Bet v 1: 71.4%, Mal d 1: 74.8%, Cor a 1: 86.4%) (Table 2).

Discussion

This study evaluated the production kinetics, cross-reactivity, IgE-blocking activity and specificity of Bet v 1-specific IgG4 Abs induced by BP-SIT performed for up to 36 months. Monitoring Bet v 1-, Mal d 1-, and Cor a 1-reactive Ab levels in 42 individuals revealed an overall significant increase in allergen-specific IgG4 and a decrease in IgE during the course of treatment. Notably, Bet v 1-specific IgE levels were significantly reduced already after 12 months of BP-SIT, whereas a significant reduction in food allergen-reactive IgE started at later time points. Bet v 1- and food allergen-reactive IgG4 appeared simultaneously after 3 months of BP-SIT. To study whether food allergen-reactive IgG4 Abs blocked IgE allergen binding, we employed different experimental approaches, namely FAB assays, BAT, and IgE-mediated allergen presentation to specific T cells. Concordantly, all results showed that post-SIT sera containing Bet v 1- and food allergen-reactive IgG4 Ab inhibited IgE binding to the major birch pollen allergen and its food homologs. Previous studies have attributed most of the inhibitory capacity in post-SIT sera to IgG4 Ab (25). Accordingly, the blocking activity of individual sera correlated with allergen-specific IgG4 levels in our experimental setups, indicating that BP-SIT can induce IgG4 Abs able to inhibit IgE binding to Bet v 1, Mal d 1, and Cor a 1. However, at early time points of SIT, we noticed blocking activity despite still low allergen-specific IgG4 levels. It has been suggested that IgE shows a higher tendency toward cross-reactivity than IgG4 (26). Accordingly, we calculated the individual ratios of Mal d 1/Bet v 1 and Cor a 1/Bet v 1 for IgE at m0 and IgG4 at m12 and m36, respectively, and found preliminary evidence

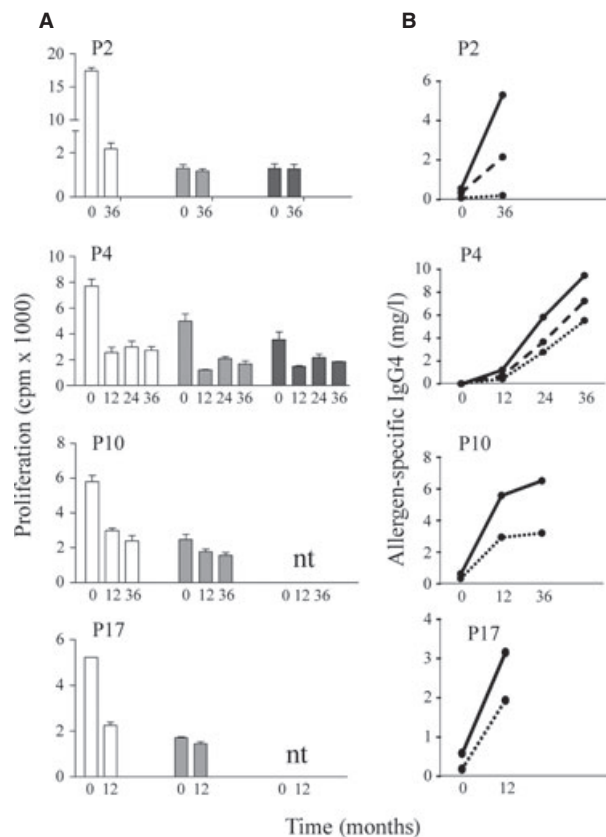


Figure 3 Post-BP-SIT sera inhibit IgE-mediated allergen-induced T-cell proliferation. (A) T-cell proliferation to Bet v 1 (white bars), Mal d 1 (light gray bars), and Cor a 1 (dark gray bars) pre-incubated with sera from four patients collected before (0) and during BP-SIT. n.t., not tested, because TCL were not cross-reactive with Cor a 1. (B) IgG4 levels specific for Bet v 1 (full line), Mal d 1 (dotted line), and Cor a 1 (dashed line) in the respective sera at indicated time points.

Table 2 Summary of mimotopes, predicted IgE/IgG4 epitopes and their overlap

	Bet v 1-IgE	Bet v 1-IgG4	Mal d 1-IgE	Mal d 1-IgG4	Cor a 1-IgE	Cor a 1-IgG4
P1						
Mimotopes	TCAKATSTPPLS DPQIAYERANTS KPPSLTPHILTA DVVPASTRDHTD GTTTLNHNYSK QMSLOQRSHYTS	SIPRYHTENSP SSPSAAQWLNIG LPGRAHDPWKVP HPATSNTKYWIN FTTESGGLNPHA VEAHNQWTLGLVA YHPNGMNPYTKA APTIAYNTFMPM	DRLPGTQAVRLV YHPNGMNPYTKA SNLSWPANMKHP TYNTHPDFTYSS VEAHNQWTLGLVA	EQVPASSAQWSR MDRANLVKPSTL KFNPGNSEWQRT SATNGSLTRPVH GYSPTKDRMESA GTTTLNHNYSK	YHPNGMNPYTKA YHPNGMNPYTKA STNSPPASMRTN RSDALEYMSKLR KFNPGNSEWQRT LWAYPVYITGKW	TDPYLSTRLSLH TSPDVVAPQLTK APKSTVDSAQNL TCAKATSTPPLS
No of predicted epitopes	3	3	3	2	4	1
No of IgG4 epitopes overlapping with IgE (%)		2 (66%)		2 (100%)		1 (100%)
P2						
Mimotopes	HHFEKRWGAETL YHKSPNACDLSP YHPNGMNPYTKA NHSFDKSTLSTG TAKMQVNNMKPS	ESIHASGKMGRH ANHLSGNNYGIS GTTTLNHNYSK YHPNGMNPYTKA NDNYPATIKHSN SVDMPHPYQGRP	QLYPGTPAAERL MHNRPPTYAHGD SQPFKTWQSHDL NQAKDHHNLQRY TVEPNKLKFTQT	HGIPKHNDKDMRT YKPMADRSSAVF ACQHKYCRADFV VLIPSNFSPDKS HLHTYQRPVQFY	GNNASKDVSATR SSLHSSHEMNK MDTTRSAEKIKW VQHNTKYSVIR SEASWTKVSQLK	TSYPSYLHRTMP QNGNITALHSYV VNLEKSSSDTRH FEDHRGWAVDRM QQSWPSHLSPTK
No of predicted epitopes	4	2	4	4	2	3
No of IgG4 epitopes overlapping with IgE (%)		1 (50%)		3 (75%)		2 (66%)
P3						
Mimotopes	OHMGVSPAAAAA IFAALDYNLGRH DSHSTHKALMAH GTTTNSHNYSK YHPNGMNPYTKA SNLSWPANMKHP GTTTNSHNYSK	HMGMTKINYSAL HMGMTKINYSAL HMGMTKINYSAL LSSHKATSTRAD SLPFGPNTTIRP LESHYQASYTQ	SNLSWPANMKHP YNDMSCCMAVHH AVEQEAARHYNW YHPNGMNPYTKA WSEYDIPTQIP	EVEMKGRHLAQM YYNPSPNPRTQ TGSWATPKGVRM	HSHELQSSAKIA TAYTLTNPETGY YHPNGMNPYTKA	MADHSRHPNSSM LNAKVPLPGHKS YSINAIKNARHF NRPDASQFWLHH KLHISKDHIYPT
No of predicted epitopes	5	4	4	3	2	3
No of IgG4 epitopes overlapping with IgE (%)		3 (75%)		2 (66%)		2 (66%)
P6						
Mimotopes	VQHNTKYSEVIR KPVAYYPWTGSQ IHNPQQNQQTST EQVPASSAQWSR STNSPPASMRTN GYTSSEPRHLTL YTDIHQNTAA	YHPNGMNPYTKA IHNPQQNQQTST VPQARVHDMRP VTQEYRGNNNIS DSRPWMESWKTP	FDFPHGKLDKRD SAVEKEGYPLR AQENIYRIMAPA APCPKNMCSSTV SLAGATATAALR	IFAALDYNLGRH NTALTEDNSFQR QVFAVRDWHHVP AKAADLPMYQGN	KFNPGNSEWQRT EQHNTKYSVIR TPPKQMINTFIN TQPHQAVDVLHS SASIFSHGHARV MTFDTVSNIYKM GEAKTRLLPSQH	YHPNGMNPYTKA KFNPGNSEWQRT DPVYVKRQDRIV IQTSRGDWDMA GKMPSNPGLKSG
No of predicted epitopes	3	3	2	3	5	2
No of IgG4 epitopes overlapping with IgE (%)		2 (66%)		1 (33%)		2 (100%)
P17						
Mimotopes	TSVPLDGTLLQH AWNTRVDSLSDK TWTNDQQRSQQR SEASWTKVSQLK GDSAGGTRLYVV EHYMASTYFSPK AHRDAHASLPMH GVKDVNAMDRVT SHEPVLMIQKFK QLYPGTPAAERL	TSNNTLTFTKFQ TLETTIKHNYWL DLAKNGVMTKGF SVTVNATSLGTT KALDNRSRTQL	THLGLYQRNTMN FDFPHGKLDKRD LPGRAHDPWKVP IFAALDYNLGRH HANSYNPTKDHK YHPNGMNPYTKA HNDGQSRPMASH	VLDNGTQKHHTK NLNGSQHTVSP DVSMPVPVPPNR	ESTMNNHSYITL GEQAKTPVGSFE GTTTLNHNYSK AGLHFMKPSERN SFMNNSVRYNM SELNGQTPTMTA NSIDKTLVIPPT	KFNPGNSEWQRT FMPILSSPSTYT FTTESGGLNPHA TSRELPLVMASN TTITTHKLPVFR

Table 2 (continued)

	Bet v 1-IgE	Bet v 1-IgG4	Mal d 1-IgE	Mal d 1-IgG4	Cor a 1-IgE	Cor a 1-IgG4
No of predicted epitopes	3	3	3	2	4	2
No of IgG4 epitopes overlapping with IgE (%)		3 (100%)		2 (100%)		2 (100%)

Peptides binding both IgE and IgG4 and/or detected multiple times with different sera are underlined.

that Mal d 1-IgE has a lower tendency to cross-react than Mal d 1-IgG4, whereas Cor a 1-IgE has a higher tendency to cross-

react than Cor a 1-IgG4. However, experimental proof for this hypothesis is still needed.

Vaccination with genetically modified non-IgE-binding Bet v 1-variants was reported to induce IgG Abs that inhibit IgE recognition of Bet v 1- and Bet v 1-related food allergens due to steric hindrance after binding to new sequential epitopes not recognized by IgE (27, 28). To investigate whether Bet v 1-specific IgG4 Abs induced by administration of birch pollen extract recognized IgE epitopes, we combined competitive immunoscreening of phage-displayed 12-mer peptides with EpiSearch. This algorithm has been demonstrated to predict conformational epitopes as determined by X-ray structures of antigen–Ab complexes (18). EpiSearch has also predicted conformational epitopes on the major cockroach allergen Bla g 2 in good accordance with the results from co-crystal structural analysis (29). All our predicted IgE epitopes on the surface of Bet v 1 matched those reported by Mittag et al. (9) who employed a random phage-displayed 7-mer peptide library as well as those identified by other experimental approaches, for example by determination of the X-ray structure of an antigen–Ab complex or by employment of peptide-specific monoclonal Ab and human monoclonal IgE (7, 30–33). Hence, we proceeded to predict IgG4 epitopes on Bet v 1, Mal d 1, and Cor a 1, respectively, and calculated their potential overlap with IgE epitopes (Fig. 4). Comparing predicted binding sites of IgG4 and IgE Abs from five different individuals on all three allergens revealed that on average, 77.5% IgG4 epitopes overlapped with IgE epitopes. The high degree of overlap suggests that the majority of BP-SIT-induced IgG4 Abs recognize IgE binding areas. This hypothesis is further supported by the elution of several identical mimotopes from IgE- and IgG4-loaded beads.

In summary, our data provide evidence that not all but most patients receiving BP-SIT develop Bet v 1-specific IgG4 Abs that cross-react with homologous food allergens and block IgE allergen binding by direct epitope competition. However, our data also indicate that SIT-induced IgG4 Abs are not simply IgE epitope identical responses as more than 35% of predicted IgE epitopes were not recognized by IgG4 Abs (mean for Bet v 1: 36%, Mal d 1: 38%, Cor a 1: 37%). Thus, SIT-induced IgG4 Abs may not cover all IgE specificities. On the other hand, around 20% of IgG4 epitopes did not overlap with IgE epitopes (mean for Bet v 1: 28%, Mal d 1: 25%, Cor a 1: 13%). The induction of *de novo* IgG4-specificities has been reported for SIT with grass pollen and recently also for oral immunotherapy with peanut (34, 35). Based on our results, we conclude that the majority of SIT-induced IgG4 Abs recognize IgE epitopes, and a minority represent *de novo* specificities.

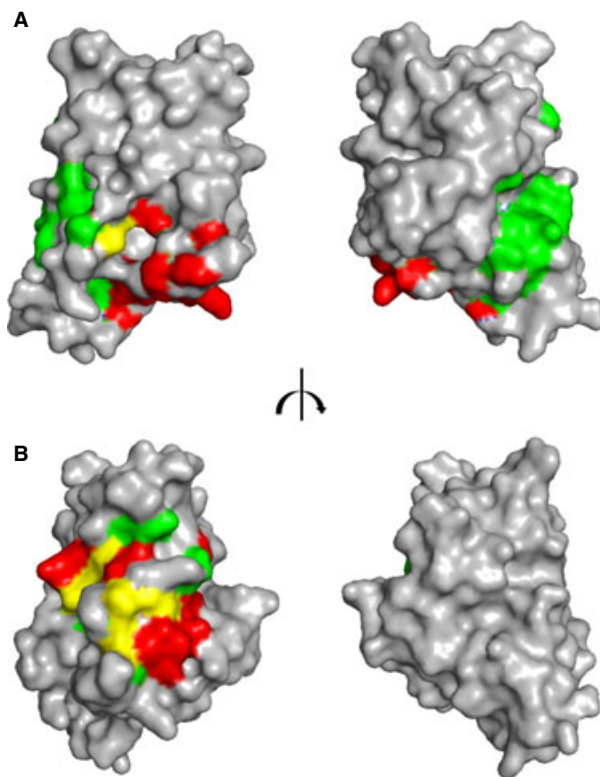


Figure 4 Distinct and overlapping epitopes on Bet v 1. Representative examples for (A) distinct epitopes: mimotope GTTTLNHNYS- A K predicting epitope Bet v 1_{N4}, Y5, N82, Y83, L95, K97, S99, K115, S117, N118, K119, H121, K134, A135, S136, K137, G140 (ASA = 902.2 Å², red) and mimotope YHPNGMNPYTKA predicting epitope Bet v 1_{K55}, P59, G61, P63, K65, Y66, G88, G89, P90, G92, A135, M139 (ASA = 498.7 Å², green). The intersection of the two epitopes (A135) is 56.8 Å² (yellow), leaving 707.9 Å² (>600 Å²) of contiguous surface area (Bet v 1_{N4}, Y5, N82, Y83, L95, K97, S99, K115, S117, N118, K119, H121, K134) for potential independent immunoglobulin binding. (B) overlapping epitopes: mimotope DVVPASTRDHTD predicting epitope Bet v 1_{D25}, D27, P31, V33, P35, S39, S40, V41, S57, P59, D69, T142, R145, A146 (ASA = 670.2 Å², red) and mimotope APTIAYNTFMPM predicting epitope Bet v 1_{F22}, I23, N28, F30, P31, P35, I56, F58, P59, M139, T142, A146, Y150 (ASA = 498.7 Å², green). The intersection of the two epitopes (P31, P35, P59, T142, T146) has 230.2 Å² ASA (yellow), leaving a largest contiguous surface area (Bet v 1_{S39}, S40, V41, S57) of 134.16 Å² (<600 Å²). Protein models (pdb 1BV1) were displayed using Polyview3D (36).

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Author contributions

B.S. and B.B. designed the experiments; B.S. and D.S. performed the experiments and analyzed the data; C.M., N.J., C.E., N.R., W.P., and R.G.W. provided patients' sera, D.B.,

J.L., and S.V. performed ImmunoCAP; B.S. and B.B. wrote the manuscript.

Conflicts of interest

The authors declare no competing financial interests.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Amino acid sequences of mimotopes and predicted IgE and IgG4 epitopes on Bet v 1, Mal d 1 and Cor a 1.

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Patient	Allergen-Antibody	mimotopes*	Amino acid residues of predicted epitopes**
P1	Bet v 1-IgE	<u>TCAKATSTPPLS</u>	T7, P63, P90, S117, K119, K129, A130, K134, A135, S136, K137, T142, L143
		DPQIAYERANTS	I23, D27, P50, I53, I56, D69, R70, D72, E73, Y81, N82, Y83, I86, S99, E101, I102, I116, S117, N118 S39, I56, S57, P59, E60, P63, Y66, E87, P90, I91, Q132, A135, S136,
		KPPSLTPIHLTA	S39, K55, I56, S57, P59, L62, P63, K65, K68, P90, I91, A135, S136
		DVVPASTRDHTD	D25, D27, P31, V33, P35, S39, S40, V41, S57, P59, D69, T142, R145, A146
		<u>GTTTLNHNYSK</u>	N4, Y5, T7, N82, Y83, L95, K97, S99, K115, S117, N118, K119, H121, K134, A135, S136, K137, G140 N4, Y5, T7, T9, K80, Y81, N82, Y83, S99, K103, K115, S117, N118, K119, S136, K137, G140, L143
		QMSLQYRSHYTS	T9, T10, R17, T142, L143, R145, S149, Y150, L152, H154, Y158
	Bet v 1-IgG4	SIPRYYHTENSP	I23, P50, I53, I56, R70, E73, Y81, N82, Y83, I86, S99, E101, I102, I116, S117, N118, S39, S40, E42, I44, I53, I56, S57, P63, Y66, R70, Y83, I86, E87, P90, I91, S136 N28, P31, S40, E42, N43, I44, E45, N47, I53, I56, S57, Y66, R70, Y83, I86, E87
		APTIAYNTFMPM	T9, F22, I23, F30, P31, P35, I56, I102, I116, M139, T142, A146, Y150 F22, I23, N28, F30, P31, P35, I56, F58, P59, M139, T142, A146, Y150 I56, F58, P59, P63, F64, Y66, P90, I91, A135, M139 F30, P31, N43, I44, I53, I56, F58, P59, F64, Y66, Y83, I86, M139 I44, I53, I56, F58, P63, F64, Y66, Y83, I86, P90, I91, A135, M139
		<u>YHPNGMNPYTKA</u>	K55, P59, G61, P63, K65, Y66, K68, G88, G89, P90, G92, A135, M139
		HPATSNTKYWIN	N4, Y5, T7, N82, Y83, P90, K97, S99, I102, K115, I116, S117, N118, K119, H121, K134, A135, S136, K137 S39, S40, I44, I53, K54, K55, I56, S57, P63, K65, Y66, K68, Y83, I86, P90, I91, K97, A135, S136
		<u>FTTESGGLNPHA</u>	T7, T9, F30, F58, L62, P63, F64, G89, P90, N118, A135, S136, E138, G140, E141, T142, L143, A146 S39, S57, F58, P59, E60, G61, L62, P63, F64, E87, G88, G89, P90, G92, A135, S136
		<u>LPGRAHDPWKVP</u>	V41, K54, K55, L62, P63, K65, V67, K68, D69, R70, V85, G88, G89, P90, G92, L95, K97, A135
		<u>SSPSAAQWLNIG</u>	S39, I56, S57, P59, G61, L62, P63, G88, G89, P90, I91, G92, Q132, A135, S136
		<u>VEAHNQWTGLVA</u>	G1, V2, N4, G92, T94, L95, E96, N118, H121, T122, H126, E127, V128, A130, E131, Q132, V133, A135
	Mal d 1-IgE	DRLPGTQAVRLV	L24, D25, A26, D27, L29, P31, P35, Q36, A37, Q40, A41, T57, A139, G141, L142, L145 L29, P31, P35, Q36, A37, Q40, A41, T57, G61, Q63, V134, G135, A139, G141, L142, L145
		<u>YHPNGMNPYTKA</u>	A26, N28, P31, A41, G46, N47, G48, G49, P50, G51, K54, K55, T57, Y66, K68, H69, Y83 A26, A41, G48, G49, P50, G51, K54, K55, T57, Y66, K68, H69, Y81, Y83, G88, K97, Y100
		<u>SNLSWPANMKHP</u>	A26, L29, P31, K32, P35, A37, A41, S115, K136, K138, A139, H140, L142, K144, L145, S148 L24, A26, N28, L29, P31, K32, P35, A37, K39, A41, K54, H69, K138, A139, L142, L145
		TYNTHPDFTYSS	N7, F9, F22, D72, S73, D75, S78, Y79, S80, Y81, S82, Y83, S99, Y100, T102, T112, S115, H118
		<u>VEAHNQWTGLVA</u>	G1, V2, T4, A90, L91, T92, T94, E96, H118, H120, T121, G123, N124, E126, E129, E130, H131, V134, G135
	Mal d 1-IgG4	<u>KFNPGNSEWQRT</u>	T4, F5, E6, N7, E8, F9, T10, F22, S80, S82, E101, T102, K103, T112, K114, S115, G135, K136, K138
		MDRANLVKPSTL	N7, A26, D72, S73, D75, S78, S80, S82, S99, T102, K103, V105, T112, K114, S115, K136, A139, L142
		<u>GTTTLNHNYSK</u>	T4, N7, R70, D72, S73, S80, S82, L85, D89, K97, S99, T102, K103, K114, S115, K136, K138, A139 T4, N7, H69, S73, S80, Y81, S82, Y83, L85, K97, S99, Y100, T102, K103, K114, S115, H118, H120, G135, K136, K138, A139

P2		GYSPTKDRMESA	A26, D27, A41, E42, E45, G48, G49, P50, G51, K54, K55, T57, Y66, K68, R70, D72, Y81, S82, Y83, E87, G88, D89, K97, S99, Y100
		SATNGSLTRPVH	A26, G48, G49, P50, G51, H69, R70, S73, S80, S82, L85, S99, T102, S115, H118, A139 V2, T4, H69, R70, S82, L85, G88, A90, L91, T92, S99, H118, H120, T121, H131, G135
		MDRANLVKPSTL	L24, D25, A26, D27, N28, L29, P31, K32, P35, A37, K39, A41, K54, T57, K138, A139, L142, L145
			A26, P31, P35, Q36, A37, A41, S112, E137, A139, E147, S148
		EQVPASSAQWSR	P31, P35, Q36, A37, Q40, A41, E42, E60, S62, Q63, V134 E12, P14, P15, S16, R17, E76, A77, S78, S80, V105, A106
	Cor a 1-IgE	YHPNGMNPYTKA	A26, P31, H37, T39, A41, N43, K54, K55, T57, A59, N62, K65, Y66, M67, K68, H69, K70, Y83, G88, G89, Y100, K140
		STNSPPASMRTN	A26, P31, T39, S40, A41, N43, T57, A59, N62, M67 A26, N28, P31, P35, T39, S40, A41, T57, A59, A147 A26, N28, P31, P35, T39, S40, A41, N43, T57, M67
		KFNPGNSEWQRT	P31, T39, S40, E42, N43, K54, K55, T57, F58, N62, F64, K65, K68, K70, E87, G88, G89, K140 P31, P35, Q36, T39, S40, E42, K55, T57, F58, E60, G61, N62, E63, F64, K65, G88, G89, K140 E42, K54, K55, T57, F58, N62, E63, F64, K65, K68, K70, E87, G88, G89, P90, G92, K97, K140 F3, K68, K70, E72, E87, G88, G89, P90, G92, T94, E96, K97, S99, E101, T118, K120, E133, G137
		LWAYPVIYTGKW	A26, I30, P31, T39, A41, L44, I53, K54, K55, I56, T57, A59, K65, Y66, K68, K70, Y83, I86, G88, G89, Y100, K140, L144
		RSDALEYMSKLR	Y5, E6, D7, A26, M67, K70, E72, E73, K80, Y81, Y83, K97, S99, Y100, E101, K103, K116, K12, Y121, A141
	Cor a 1-IgG4	TSPDVVAPQLTK	L24, D25, A26, D27, P31, K32, V33, P35, Q36, T39, S40, A41, K54, K140, A141, L144, K146, A147
		APKSTVDSAQNL	L24, D25, A26, D27, N28, P31, K32, V33, P35, Q36, T39, S40, A41, K54, K140, A141, L144, K146, A147
		ICAKATSTPPLS	A26, P31, K32, P35, T39, S40, A41, L44, K54, K55, T57, K68, L144
		TDPYLSTRLSLH	D27, P31, H37, T39, S40, L44, T57, Y66, H69, Y83, Y10 , L144 P15, L24, D25, P50, H69, D75, H76, Y81, Y83, S99, Y100, T118
	Bet v 1-IgE	HHFEKRWGAETL	K55, F58, E60, G61, L62, F64, K65, K68, E87, G88, G89, G92, A135
		YHKSPNACDLSP	N4, Y5, N82, Y83, P90, L95, K97, S99, K115, S117, N118, K119, H121, K134, A135, S136, K137 K20, L24, D27, P50, D69, D72, D75, H76, N78, K80, Y81, N82, Y83, S99, K103, K115, S117
		YHPNGMNPYTKA	K55, P59, G61, P63, K65, Y66, K68, G88, G89, P90, G92, A135, M139
		NHSFDKSTLSTG	T7, T9, F22, L24, D72, D75, N78, F79, K80, N82, S99, K103, K115, S117, N118, S136, G140, L143 F22, L24, D25, D27, N28, F30, K32, S40, N43, G46, N47, G48, G49, K54, K55, S57, D69, L143 G1, F3, G88, G89, G92, D93, T94, L95, K97, S99, N118, K119, H121, T122, K123, G124, H126, K129 K20, F22, L24, D27, G48, G49, D69, D72, D75, H76, N78, F79, K80, N82, S99, K10 , K115, S117
		TAKMQVNNMKPS	S39, K55, S57, P59, P63, K65, V67, K68, V85, P90, Q132, A135, S136, M139
	Bet v 1-IgG4	ESIHASGKMGRH	S39, K55, I56, S57, E60, G61, K65, K68, E87, G88, G89, I91, G92, A135, S136, M139
		ANHLSGNNYGIS	S39, S40, I44, I53, I56, S57, L62, Y66, Y83, I86, G88, G89, I91, G92, L95, A135, S136 N43, I44, G48, G49, G51, I53, I56, S57, Y66, Y81, N82, Y83, I86, G88, G89, I91, S99
		GTTTLNHNYSK	N4, Y5, T7, N82, Y83, L95, K97, S99, K115, S117, N118, K119, H121, K134, A135, S136, K137, G140 N4, Y5, T7, T9, K80, Y81, N82, Y83, S99, K103, K115, S117, N118, K119, S136, K137, G140, L143
		YHPNGMNPYTKA	K55, P59, G61, P63, K65, Y66, K68, G88, G89, P90, G92, A135, M139
		NDNYPATIKHSN	I23, D27, P50, I53, K54, I56, K68, D69, D72, K80, Y81, N82, Y83, I86, K97, S99, I102, I116, S117, N118, K119

		<u>SVDMHPHYGQRP</u>	S39, S40, V41, S57, P63, Y66, V67, D69, R70, Y83, V85, G88, G89, P90, G92, S136, M139
		<u>QLYPGTPAAERL</u>	A26, A41, E42, L44, E45, G48, G49, P50, G51, T57, Y66, R70, Y81, Y83, L85, E87, G88, L91, Y100
		<u>MHNRPTTYAHGD</u>	A26, D27, A41, G48, G49, P50, G51, T57, Y66, H69, R70, D72, Y81, Y83, G88, D89, Y100
	Mal d 1-IgE	<u>NQAKDHHNLQRY</u>	L24, D25, A26, D27, N28, L29, K32, Q36, A37, K39, Q40, A41, K54, H69, K138, A139, L142, L145, Y149 K54, K55, Y64, Y66, K68, H69, R70, D72, Y83, L85, D89, A90, L91, K97, Y100, H118, H120, H131, K138 A26, D27, A37, K39, Q40, A41, L44, K54, K55, Y64, Y66, K68, H69, R70, Y83, L85, D89, K138, L142
		<u>TVEPNKCLKFTQT</u>	F22, L24, N28, L29 P31, K32, P35, Q36, K39, Q40, E42, K54, T57, F58, K138, L142, L145 E6, N7, E8, F9, T10, E12, F22, T102, K103, V105, T112, K114, K136, E137, L142, K144, E147
		<u>HGIPKHNMKDMRT</u>	D27, I30, I43, G48, G49, P50, G51, I53, K54, K55, I56, T57, K68, H69, R70, D72, I86, G88, D89, K97, I98
		<u>YKPMADRSSAVF</u>	F9, F22, A26, D72, S73, D75, S78, Y79, S80, Y81, S82, Y83, S99, Y100, K103, V105, K114, S115, K136, A139
	Mal d 1-IgG4	<u>ACQHKYCRADVF</u>	K54, K55, Y64, Y66, K68, H69, R70, D72, Y83, D89, A90, K97, Y100, H118, H120, H131, K138 A26, D27, A37, K39, Q40, A41, K54, K55, F58, Y64, Y66, K68, H69, R70, Y83, D89, K138
		<u>VLIPSNFSPDKS</u>	D27, I30, I43, L44, P50, I53, K54, K55, I56, K68, D72, S82, L85, I86, D89, L91, K97, I98, S99
		<u>HLHTYQRPVQFY</u>	V2, Y3, T4, F5, H69, R70, Y83, L85, L91, T92, Y100, H118, H120, T121, H131
		<u>GNNASKDVSATR</u>	A26, D27, T39, S40, A41, N43, K54, K55, T57, A59, N62, K65, K68, K70, G88, G89, K140 D25, A26, D27, N28, S40, A41, N43, G46, N47, G48, G49, G51, K54, K55, T57, K68, K70
	Cor a 1-IgE	<u>SSLHSSHHEMNK</u>	H37, S40, E42, N43, L44, K54, K55, N62, K65, M67, K68, H69, K70, E87, K140, L144
		<u>MDTTRS AEKIKW</u>	E6, D7, A26, I56, M67, K70, E72, E73, K80, I86, K97, I98, S99, E101, I102, K103, K116, I117, T118, K120, I134, A141
		<u>VQHNTKYSVVIR</u>	Y5, I56, H69, K70, K80, Y81, Y83, I86, K97, I98, S99, Y100, I102, K103, K116, I117, T118, K120, Y121, H122, I134
		<u>SEASWTKVSQLK</u>	A26, T39, S40, A41, E42, L44, K54, K55, T57, A59, K65, K68, K70, E87, K140, L144
		<u>TSYPSYLHRTMP</u>	Y5, M67, H69, Y83, P90, L91, T94, S99, Y100, T118, Y121, H122 P31, H37, T39, S40, L44, T57, Y66, M67, H69, Y83, Y100, L144
	Cor a 1-IgG4	<u>QNGNITALHSYV</u>	A26, A41, N43, L44, G48, G51, I53, I56, T57, Y66, H69, Y81, Y83, I86, G88, G89, L91, I98, S99, Y100, I102 A26, I30, H37, T39, S40, A41, N43, L44, I53, I56, T57, A59, N62, Y66, H69, Y83, I86, G88, G89, Y100, L144
		<u>VNLEKSSSDTRH</u>	D27, H37, T39, S40, E42, N43, L44, K54, K55, T57, N62, K65, K68, H69, K70, E87, K140, L144
		<u>QQSWPSHLSPTK</u>	P31, H37, T39, S40, L44, K54, K55, T57, K65, K68, H69, K70, K140, L144
		<u>FEDHRGWAVDRM</u>	A9, F22, A26, D27, V33, H37, A41, F58, G137, E139, A141, A142, G143, F145, A147, A150 D7, E8, A9, V12, F22, A26, V33, H37, G137, E139, A141, A142, G143, F145, A147, A150
		<u>IFAALDYNLGRH</u>	A16, R17, F22, I23, L24, D25, D27, N28, F30, G48, G49, D75, H76, N78, F79, Y81, N82, Y83, I102, I116, L143, Y158
		<u>YHPNGMNPYTKA</u>	K55, P59, G61, P63, K65, Y66, K68, G88, G89, P90, G92, A135, M139
	Bet v 1-IgE	<u>DSHSTHKALMAH</u>	D93, T94, L95, K119, H121, T122, K123, H126, K129, A130, K134, A135, S136 T7, L95, K97, S99, K115, S117, K119, H121, K134, A135, S136, K137, M139
		<u>SNLSWPANMKHP</u>	N4, P90, L95, N118, K119, H121, K123, H126, K129, A130, K134, A135, S136 K54, K55, K68, N82, P90, L95, K97, S99, N118, K119, H121, S136, M139 P63, P90, S117, N118, K119, K129, A130, K134, A135, S136, K137, M139, L143 N4, P90, L95, S117, N118, K119, K129, A130, K134, A135, S136, K137, M139
		<u>QHMGVSPAAAAA</u>	Q36, P63, V67, V85, G89, P90, Q132, V133, A135, S136, M139, G140, A146
		<u>GTTTSNHNYS AK</u>	N4, Y5, T7, N82, Y83, K97, S99, K115, S117, N118, K119, H121, K134, A135, S136, K137, G140

	Bet v 1-IgG4	SLPFGPNTTIRP	S39, I56, S57, F58, P59, G61, L62, P63, F64, G88, G89, P90, I91, G92, S136
		<u>HMGMTKINYSAL</u>	S39, K55, I56, S57, G61, L62, K65, Y66, K68, G88, G89, I91, G92, A135, S136, M139
			S39, S40, I44, I53, K54, K55, I56, S57, L62, K65, Y66, K68, Y83, I86, G88, G89, I91, G92, L95, K97, A135, S136, M139
			N4, Y5, T7, T9, I23, K80, Y81, N82, Y83, S99, I102, K103, I113, K115, I116, S117, N118, K119, S136, K137, M139, G140, L143
		LESHYTQASYTQ	T9, T10, E141, T142, L143, A146, E148, S149, Y150, L152, A153, H154, A157, Y158
	Mal d 1-IgE	LSSHATSTRAD	D93, T94, L95, K119, H121, T122, K123, H126, K129, A130, K134, A135, S136
			R17, D25, K32, L143, R145, A146, S149, L152, A153, H154, S155, D156, A157
		WSEYDIPTQIP	P15, P50, D72, S73, I74, D75, E76, S78, Y79, S80, Y81, S82, Y83, Y100, E101, T102, S115, I116
		<u>YHPNGMNPYTKA</u>	A26, A41, G48, G49, P50, G51, K54, K55, T57, Y66, K68, H69, Y81, Y83, G88, K97, Y100
			A26, N28, P31, A41, G46, N47, G48, G49, P50, G51, K54, K55, T57, Y66, K68, H69, Y83
	Mal d 1-IgG4	AVEQEAAARHYNW	N7, A37, Q63, Y64, A90, Y100, E129, E130, H131, V134, E137, A139, H140
		<u>SNLSWPANMKHP</u>	A26, L29, P31, K32, P35, A37, A41, S115, K136, K138, A139, H140, L142, K144, L145, S148
			L24, A26, N28, L29, P31, K32, P35, A37, K39, A41, K54, H69, K138, A139, L142, L145
		TGSWATPKGVRM	A26, G48, G49, P50, G51, K54, K68, R70, S73, S80, S82, K97, S99, T102, S115, A139
			T10, P14, P15, S16, R17, K20, A77, S78, S80, K103, V105, A106, G108, G110, T112, K114
	Cor a 1-IgE	YYNPSPNPRTQ	T10, P14, P15, S16, R17, Y79, S109, T112, P154, Y157, N158
			P14, P15, S16, R17, S78, Y79, S80, Y81, S82, T102, T112
		EVEMKGRHLAQM	L29, K32, Q36, A37, K39, Q40, A41, G61, Q63, V134, G135, E137, K138, A139, H140, G141, L142, K144, L145
		HSHELQSSAKIA	E6, A26, I56, H69, K70, E72, E73, K80, I86, K97, I98, S99, E101, I102, K103, K116, I117, K120, H122, I134, A141
		TAYTLTNPETGY	L24, A26, N28, P31, A41, E42, N43, L44, E45, G46, N47, G48, G49, G51, T57, Y66, Y83, E87
	Cor a 1-IgG4	<u>YHPNGMNPYTKA</u>	A26, P31, H37, T39, A41, N43, K54, K55, T57, A59, N62, K65, Y66, M67, K68, H69, K70, Y83, G88, G89, Y100, K140
		KLHISKDHIYPT	D27, I30, P31, H37, T39, S40, L44, I53, K54, K55, I56, T57, K65, Y66, K68, H69, K70, Y83, I86, Y100, K140, L144
			Y5, D7, I56, H69, K70, K80, Y81, Y83, I86, K97, I98, S99, Y100, I102, K103, K116, I117, T118, K120, Y121, H122, I134
		NRPDSAQFWLHH	F22, L24, D25, A26, D27, N28, P31, P35, Q36, H37, S40, A41, F58, A141, L144, F145, A147
		YSINAIKNARHF	A26, I30, H37, S40, A41, N43, I53, K54, K55, I56, F58, A59, N62, F64, K65, Y66, K68, H69, K70, Y83, I86, Y100, K140
P6	Bet v 1-IgE	LNAKVPLPGHKS	A26, P31, H37, S40, A41, N43, L44, K54, K55, A59, N62, K65, K68, H69, K70, G88, G89, K140, L144
		MADHSRHPNSSM	D25, A26, D27, N28, P31, P35, H37, S40, A41, A147, H155, A158
		VQHNTKYSEVIR	N4, Y5, E6, T7, E8, T9, I23, K80, Y81, N82, Y83, S99, E101, I102, K103, V105, I113, K115, I116, S117, N118, K119, V133, S136, K137, E141
		KPVAYYPWTGSQ	S39, K55, S57, P59, G61, P63, K65, Y66, V67, K68, V85, G88, G89, P90, G92, Q132, A135, S136
		<u>IHNPOONQOTST</u>	N4, P90, I91, T94, N118, H121, T122, H126, Q132, S136
			P63, I86, P90, I91, T94, N118, H121, T122, Q132, S136
			I86, P90, I91, T94, S99, N118, H121, T122, H126, Q132
			N4, I86, P90, I91, T94, S99, N118, H121, T122, H126
		<u>EQVPASSAQWSR</u>	S39, S57, P59, E60, P63, V67, V85, E87, P90, Q132, A135, S136
		<u>STNSPPASMRTN</u>	N28, P31, P35, S39, S40, S57, P59, M139, T142, R145, A146
		GYTSSEPRLHTL	P59, E60, G61, L62, P63, Y66, G88, G89, P90, E131, S136, E138
			S39, S40, S57, P59, E60, G61, L62, P63, Y66, E87, G88, G89, P90
			S39, S57, P59, E60, G61, L62, P63, Y66, E87, G88, G89, P90, G92, S136
			P35, S39, S57, P59, E60, G61, L62, P63, Y66, G89, E138, T142

	YTDIHQNTAA	N4, Y5, I91, D93, T94, E96, N118, H121, T122, H126, E127, A130, E131, Q132, A135
Bet v 1-IgG4	<u>YHPNGMNPYTKA</u>	K55, P59, G61, P63, K65, Y66, K68, G88, G89, P90, G92, A135, M139
	<u>IHN PQQNQQTST</u>	N4, P90, I91, T94, N118, H121, T122, H126, Q132, S136 P63, I86, P90, I91, T94, N118, H121, T122, Q132, S136, I86, P90, I91, T94, S99, N118, H121, T122, H126, Q132 N4, I86, P90, I91, T94, S99, N118, H121, T122, H126
	VPQARWHDMPRP	D25, D27, P31, V33, P35, Q36, V41, P59, D69, M139, R145, A146
	VTQEYRGNNNIS	V41, E42, N43, I44, E45, G48, G49, G51, I53, I56, S57, Y66, V67, R70, Y81, N82, Y83, V85, I86, E87, G88, G89, I91, S99 S39, I56, S57, E60, G61, Y66, V67, V85, E87, G88, G89, I91, G92, Q132, S136
	DSRPWMESWKTP	E6, T7, P63, P90, S117, K119, K129, E131, K134, S136, K137, E138, M139, E141, T142 D27, P31, S39, S40, E42, K54, K55, S57, P59, K65, K68, D69, R70, E87, M139
Mal d 1-IgE	<u>FDFPHGKLDKRD</u>	D27, L44, G48, G49, P50, G51, K54, K55, K68, H69, R70, D72, L85, G88, D89, L91, K97
	SAVEKEGYPLR	A26, A41, E42, L44, E45, G48, G49, P50, G51, K54, K55, Y66, K68, R70, Y81, S82, Y83, L85, E87, G88, L91, K97, S99, Y100
	AQENIYRIMAPA	A26, N28, I30, P31, Q40, A41, E42, I43, E45, N47, P50, I53, I56, Y66, R70, Y83, I86, E87
	APCPKNMCSSTV	T10, P14, P15, S16, K20, A77, S78, S80, K103, V105, A106, C107, T112, K114
	SLAGATATAALR	A26, G48, G49, G51, R70, S73, S80, S82, L85, S99, T102, S115, A139
Mal d 1-IgG4	<u>IFAALDYNLGRH</u>	A26, D27, I30, A41, I43, L44, G48, G49, G51, I53, I56, Y66, H69, R70, D72, Y81, Y83, L85, I86, G88, D89, L91, I98, Y100
	NTALTEDNSFQR	E6, N7, E8, F9, F22, A26, D72, S73, D75, S78, S80, S82, S99, E101, T102, T112, S115, A139, L142
	QVFAVRDWHHVP	F22, D25, A26, D27, P31, P35, Q36, A37, Q40, A41, F58, H69, A139
	AKAADLPMYGQN	L24, D25, A26, D27, N28, L29, P31, K32, P35, Q36, A37, K39, Q40, A41, K54, K138, A139, G141, L142, L145, Y149
		A26, D27, A41, L44, G48, G49, P50, G51, K54, K55, Y66, K68, D72, Y81, Y83, L85, G88, D89, L91, K97, Y100
Cor a 1-IgE	<u>KFNPGNSEWQRT</u>	E42, K54, K55, T57, F58, N62, E63, F64, K65, K68, K70, E87, G88, G89, P90, G92, K97, K140 F3, K68, K70, E72, E87, G88, G89, P90, G92, T94, E96, K97, S99, E101, T118, K120, E133, G137 P31, T39, S40, E42, N43, K54, K55, T57, F58, N62, F64, K65, K68, K70, E87, G88, G89, K140 P31, P35, Q36, T39, S40, E42, K55, T57, F58, E60, G61, N62, E63, F64, K65, G88, G89, K140
	EQHNTKYSVVIR	Y5, E6, I56, H69, K70, E72, E73, K80, Y81, Y83, I86, K97, I98, S99, Y100, E101, I102, K103, K116, I117, T118, K120, Y121, H122, I134
	TPPKQMINTFIN	I30, P31, T39, N43, I53, K54, K55, I56, T57, F58, N62, F64, K65, M67, K68, K70, I86, K140
	<u>TQPHQAVDVLHS</u>	D25, A26, D27, P31, V33, P35, Q36, H37, T39, S40, A41, L144, A147, H155, A158 L24, D25, A26, D27, P31, V33, P35, Q36, H37, T39, S40, A41, A141, L144, A147 D25, A26, D27, P31, V33, P35, Q36, H37, T39, S40, A41, T57, A59, L144, A147
	SASIFSHGHARV	V12, A16, R17, F22, H76, A77, F79, I102, A105, A106, A107, H109, G110, G111, I114
	MTFDTVSNIYKM	D27, I30, T39, S40, N43, I53, K54, K55, I56, T57, F58, N62, F64, K65, Y66, M67, K68, K70, Y83, I86, Y100, K140
	GEAKTRLLPSQH	A26, P31, H37, T39, S40, A41, E42, L44, K54, K55, T57, A59, K65, K68, H69, K70, E87, G88, G89, K140, L144
	<u>KFNPGNSEWQRT</u>	E42, K54, K55, T57, F58, N62, E63, F64, K65, K68, K70, E87, G88, G89, P90, G92, K97, K140 F3, K68, K70, E72, E87, G88, G89, P90, G92, T94, E96, K97, S99, E101, T118, K120, E133, G137 P31, T39, S40, E42, N43, K54, K55, T57, F58, N62, F64, K65, K68, K70, E87, G88, G89, K140 P31, P35, Q36, T39, S40, E42, K55, T57, F58, E60, G61, N62, E63, F64, K65, G88, G89, K140
	DPVYVKRQDRIV	Y5, D7, I56, K70, K80, Y81, Y83, I86, K97, I98, Y100, I102, K103, K116, I117, K120, Y121, I134

		IQTSRGDWTDMA	A26, D27, A41, G48, G51, I53, I56, T57, M67, I86, G88, G89, I98, S99, I102 D25, A26, D27, I30, S40, A41, G46, G48, G49, G51, I53, I56, T57, M67, I86
		GKMPSNPGLKSG	L24, N28, P31, S40, N43, L44, G46, N47, G48, G49, G51, K54, K55, M67, K68, K70
		YHPNGMNPYTKA	A26, P31, H37, T39, A41, N43, K54, K55, T57, A59, N62, K65, Y66, M67, K68, H69, K70, Y83, G88, G89, Y100, K140
P17	Bet v 1-IgE	TSVPLDGTLLQH	S39, S57, P59, G61, L62, P63, V67, V85, G88, G89, P90, G92, Q132, S136 G1, V2, V67, V85, G88, G89, P90, G92, D93, T94, L95, S99, H121, T122, G124, H126, V128, Q132, V133
		AWNTRVDSLSDK	V2, N4, T7, N82, V85, L95, K97, S99, K115, S117, N118, K119, V128, V133, K134, A135, S136, K137
		TWTNDQQRSLQR	D25, D27, N28, Q36, S39, S40, S57, D69, T142, L143, R145
		<u>SEASWTKVSQ</u> LK	E6, T7, V67, V85, S117, K119, V128, K129, A130, E131, Q132, V133, K134, A135, S136, K137, E138, E141, T142, L143 S39, K55, S57, E60, L62, K65, V67, K68, V85, E87, Q132, A135, S136
		GDSAGGTRLYVV	S39, S40, V41, S57, L62, Y66, V67, D69, R70, Y83, V85, G88, G89, G92, L95, A135, S136 S39, S57, G61, L62, Y66, V67, V85, G88, G89, G92, A135, S136
		EHYMASTYFSPK	S39, K55, S57, F58, P59, E60, P63, F64, K65, Y66, K68, E87, P90, A135, S136, M139
		AHRDAHASLPMH	D25, D27, P31, P35, L143, R145, A146, S149, L152, A153, H154, A157
		GVKDVNAMDRVT	D27, V41, N43, G48, G49, G51, K54, K55, V67, K68, D69, R70, D72, N82, V85, G88, G89, K97,
		SHEPVLMIQKFK	S39, K55, I56, S57, F58, P59, E60, L62, P63, F64, K65, V67, K68, V85, E87, P90, I91, Q132, S136, M139,
		<u>QLYPGTPAAER</u> L	P59, E60, G61, L62, P63, Y66, G88, G89, P90, E131, Q132, A135, E138, P59, E60, G61, L62, P63, Y66, E87, G88, G89, P90, G92, Q132, A135,
	Bet v 1-IgG4	TSNNTLTFTKFQ	F3, N4, T7, N82, L95, K97, S99, K115, S117, N118, K119, Q132, K134, S136, K137, T7, T9, F22, L24, N78, F79, K80, N82, S99, K103, K115, S117, N118, S136, L143, N4, T7, T9, F22, K80, N82, S99, K103, K115, S117, N118, K119, S136, K137, L143,
		TLETTIKHNYWL	N4, Y5, E6, T7, E8, T9, I23, K80, Y81, N82, Y83, E101, I102, K103, I113, K115, I116, N118, K119, K137, E141, L143,
		DLAKNGVMTKGF	K55, F58, G61, L62, F64, K65, V67, K68, V85, G88, G89, G92, A135, M139,
		SVTVNATSLGTT	T7, V67, V85, G89, S117, N118, V128, A130, V133, A135, S136, G140, T142, L143, T7, T9, L62, V67, V85, G89, N118, V133, A135, S136, G140, T142, L143, A146,
		KALDNRWSRTQL	N4, T7, N82, L95, K97, S99, K115, S117, N118, K119, Q132, K134, A135, S136, K137, T7, T9, L24, D72, D75, N78, K80, N82, S99, K103, K115, S117, N118, S136, L143,
		<u>LPGRAHDPWKVP</u>	A26, D27, A41, L44, G48, G49, P50, G51, K54, K55, K68, H69, R70, D72, L85, G88, D89, L91, K97,
	Mal d 1-IgE	<u>FDFPHGKLDKRD</u>	D27, L44, G48, G49, P50, G51, K54, K55, K68, H69, R70, D72, L85, G88, D89, L91, K97,
		THLGLYQRNTMN	Y3, T4, H69, R70, Y83, L85, G88, L91, T92, Y100, H118, H120, T121, H131, G135,
		<u>IFAALDYNLGRH</u>	A26, D27, I30, A41, I43, L44, G48, G49, G51, I53, I56, Y66, H69, R70, D72, Y81, Y83, L85, I86, G88, D89, L91, I98, Y100,
		HANSYNPTKDHK	T4, N7, H69, D72, S73, S80, Y81, S82, Y83, D89, K97, S99, Y100, T102, K103, K114, S115, H118, H120, K136, K138, A139,
		<u>YHPNGMNPYTKA</u>	A26, A41, G48, G49, P50, G51, K54, K55, T57, Y66, K68, H69, Y81, Y83, G88, K97, Y100,
			A26, N28, P31, A41, G46, N47, G48, G49, P50, G51, K54, K55, T57, Y66, K68, H69, Y83,
		HNDGQSRPMASH	A26, D27, G48, G49, P50, G51, H69, R70, D72, S73, S80, S82, S99, S115, H118, A139,
	Mal d 1-IgG4	VLDNGTQKHTHK	G1, V2, T4, D89, L91, T92, D93, T94, H118, H120, T121, K122, G123, N124, K128, H131, K133, V134, G135,
		<u>NLNGSQHTVSP</u> I	G48, G49, P50, G51, I53, I56, H69, S73, I74, S80, S82, L85, I86, I98, S99, T102, S115, I116, H118, V2, T4, H69, S82, L85, I86, G88, L91, T92, I95, I98, S99, I116, H118, H120, T121, I127, H131, G135,

	DVSMVPVPPPNR	P15 , P50 , D72 , S73 , D75 , S78 , S80 , S82 , V105 , S115 ,
		P50 , R70 , D72 , S73 , D75 , S78 , S80 , S82 , S99 , S115 ,
Cor a 1-IgE	ESTMNNHSYITL	Y5 , E6 , I56 , M67 , H69 , E72 , E73 , Y81 , Y83 , I86 , I98 , S99 , Y100 , E101 , I102 , I117 , T118 , Y121 , H122 , I134 ,
	GEQAKTPVGSFE	A9 , F22 , A26 , P31 , K32 , V33 , P35 , Q36 , A41 , F58 , G137 , K138 , E139 , K140 , A141 , A142 , G143 , F145 , K146 , A147 , A150 ,
	<u>GTTTLNHNYS</u> AK	A26 , H37 , T39 , S40 , A41 , N43 , L44 , K54 , K55 , T57 , A59 , N62 , K65 , Y66 , K68 , H69 , K70 , Y83 , G88 , G89 , Y100 , K140 , L144 ,
	AGLHFMKPSE	A26 , P31 , H37 , S40 , A41 , E42 , N43 , L44 , K54 , K55 , F58 , A59 , N62 , F64 , K65 , M67 , K68 , H69 , K70 , E87 , G88 , G89 , K140 , L144 ,
	SFMNNSVR	S40 , N43 , F58 , N62 , F64 , Y66 , M67 , Y83 , Y100 ,
	SE	L24 , A26 , N28 , P31 , S40 , A41 , E42 , N43 , L44 , E45 , G46 , N47 , G48 , G49 , G51 , T57 , M67 , E87 ,
	NSIDKTLVIPPT	D27 , I30 , P31 , T39 , S40 , N43 , L44 , I53 , K54 , K55 , I56 , T57 , N62 , K65 , K68 , K70 , I86 , K140 , L144 ,
Cor a 1-IgG4		P31 , T39 , S40 , E42 , N43 , K54 , K55 , T57 , F58 , N62 , F64 , K65 , K68 , K70 , E87 , G88 , G89 , K140 ,
	<u>KFNPGNSEWQRT</u>	P31 , P35 , Q36 , T39 , S40 , E42 , K55 , T57 , F58 , E60 , G61 , N62 , E63 , F64 , K65 , G88 , G89 , K140 , E42 , K54 , K55 , T57 , F58 , N62 , E63 , F64 , K65 , K68 , K70 , E87 , G88 , G89 , P90 , G92 , K97 , K140 , F3 , K68 , K70 , E72 , E87 , G88 , G89 , P90 , G92 , T94 , E96 , K97 , S99 , E101 , T118 , K120 , E133 , G137 ,
	FMPILSSPSTYT	I30 , P31 , T39 , S40 , L44 , I53 , I56 , T57 , F58 , F64 , Y66 , M67 , Y83 , I86 , Y100 , L144 ,
	<u>FTTESGGLNPHA</u>	A26 , P31 , H37 , T39 , S40 , A41 , E42 , N43 , L44 , T57 , F58 , A59 , N62 , F64 , H69 , E87 , G88 , G89 , L144 ,
		E6 , E8 , A9 , P15 , L24 , A26 , E72 , E73 , N78 , S99 , E101 , A105 , T118 , A141 ,
	TSRELPVLMASN	A26 , P31 , T39 , S40 , A41 , E42 , N43 , L44 , T57 , A59 , N62 , M67 , E87 , L144 , L24 , A26 , N28 , P31 , S40 , A41 , E42 , N43 , L44 , E45 , N47 , T57 , M67 , E87 ,
	TTITTHKLPVFR	I30 , P31 , H37 , T39 , L44 , I53 , K54 , K55 , I56 , T57 , F58 , F64 , K65 , K68 , H69 , K70 , I86 , K140 , L144 ,

*peptides bound to both IgE and IgG4, respectively, are underlined.

**amino acids of predicted epitopes with the highest ranking are shown.