Online Supporting Information

Original Article

Prophylactic and therapeutic vaccination with carrier-bound Bet v 1 peptides lacking allergen-specific T cell epitopes reduces Bet v 1-specific T cell responses via blocking antibodies in a murine model for birch pollen allergy

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Short title: Allergen peptides lacking T cell epitopes reduce allergen-specific T cell responses

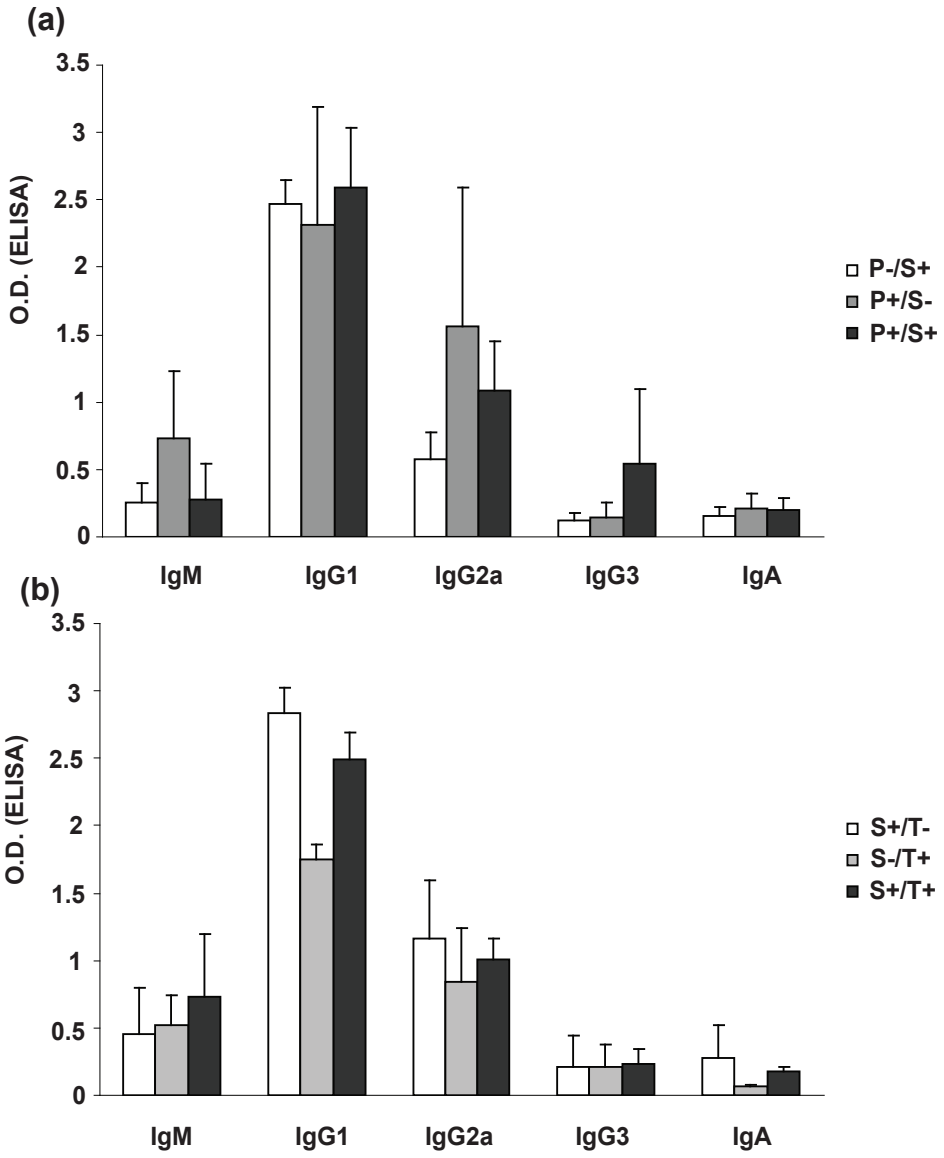
(a)
O.D. (ELISA)
3.5
3
2.5
2
1.5
1
0.5
0
IgM
IgG1
IgG2a
IgG3
IgA
P-/S+
P+/S-
P+/S+
(b)
O.D. (ELISA)
3.5
3
2.5
2
1.5
1
0.5
0
IgM
IgG1
IgG2a
IgG3
IgA
S+/T-
S-/T+
S+/T+

Figure S1

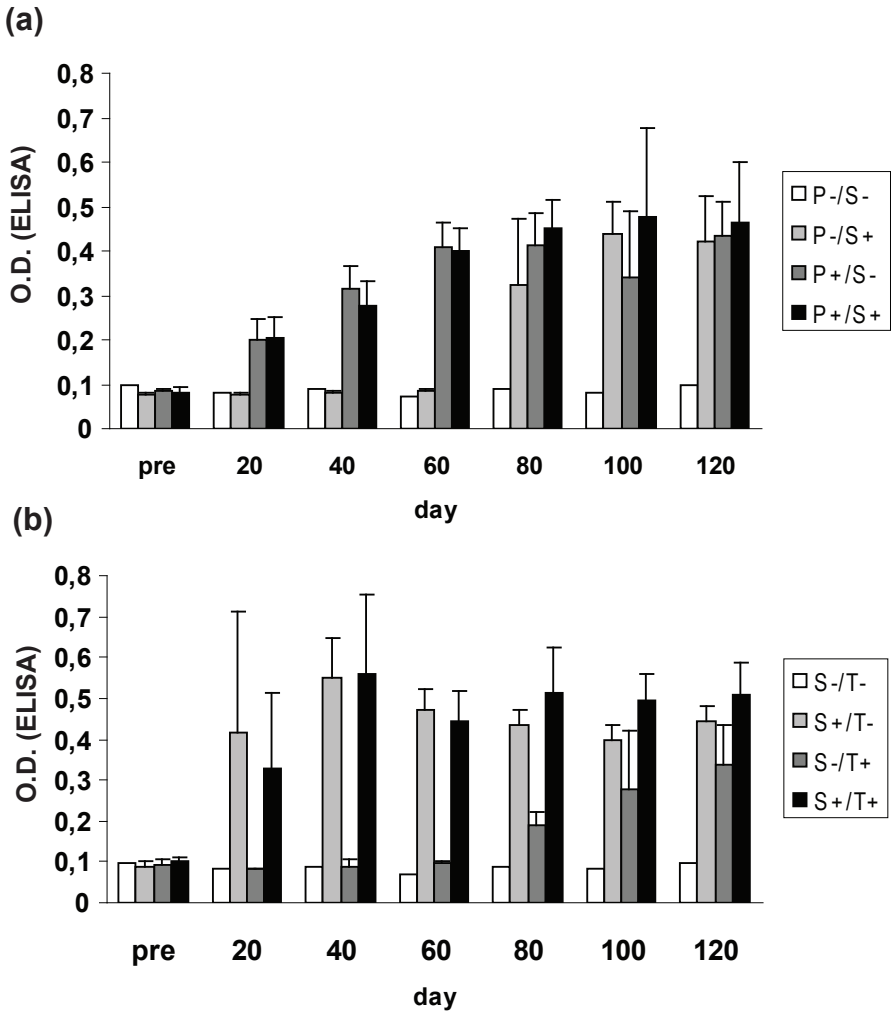
(a)
O.D. (ELISA)
0,8
0,7
0,6
0,5
0,4
0,3
0,2
0,1
0
pre
20
40
60
80
100
120
day
P-/S-
P-/S+
P+/S-
P+/S+
(b)
O.D. (ELISA)
0,8
0,7
0,6
0,5
0,4
0,3
0,2
0,1
0
pre
20
40
60
80
100
120
day
S-/T-
S+/T-
S-/T+
S+/T+

Figure S2

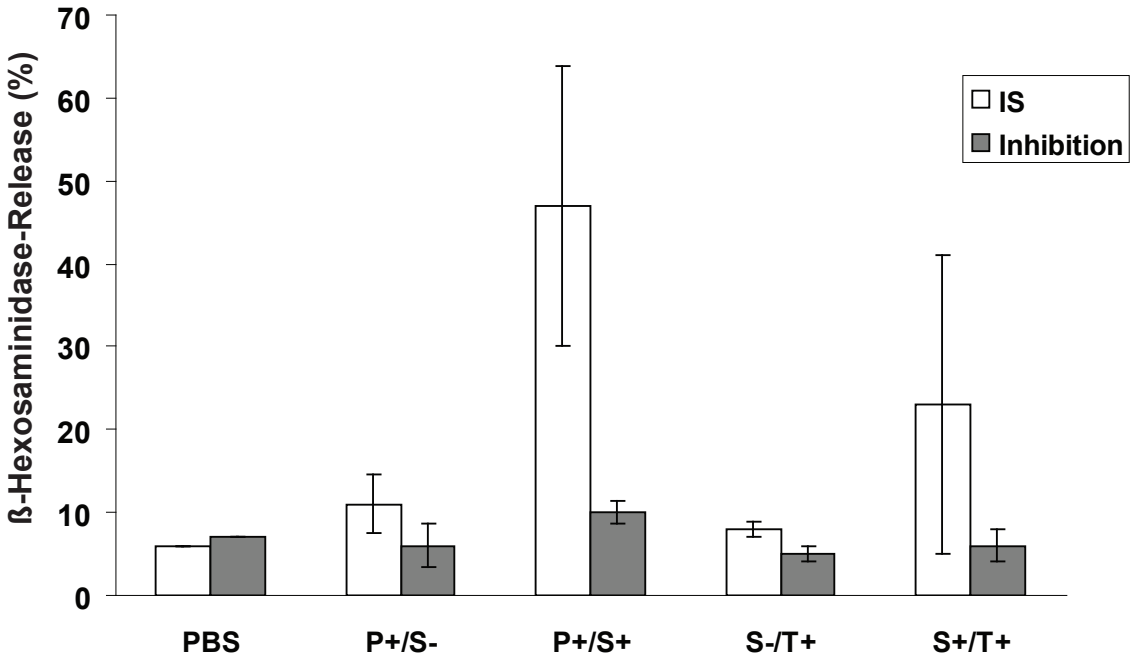
ß-Hexosaminidase-Release (%)
70
60
50
40
30
20
10
0
IS
Inhibition
PBS
P+/S-
P+/S+
S-/T+
S+/T+

Figure S3

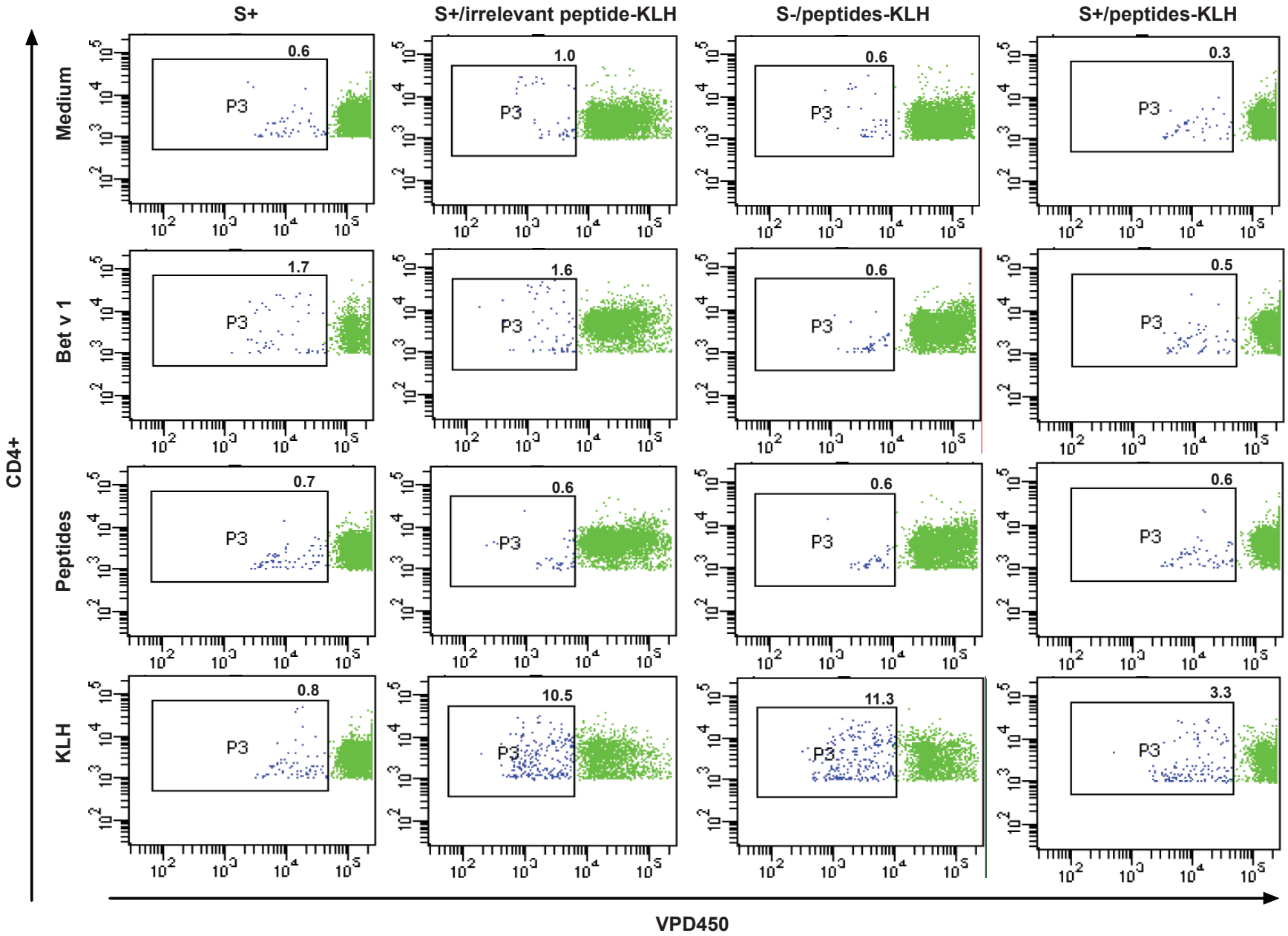
S+
S+/irrelevant peptide-KLH
S-/peptides-KLH
S+/peptides-KLH
Medium
Bet v 1
Peptides
KLH
CD4+
VPD450
P3
0.6
1.0
0.6
0.3
1.7
1.6
0.6
0.5
0.7
0.6
0.6
0.6
0.8
10.5
11.3
3.3

Figure S4

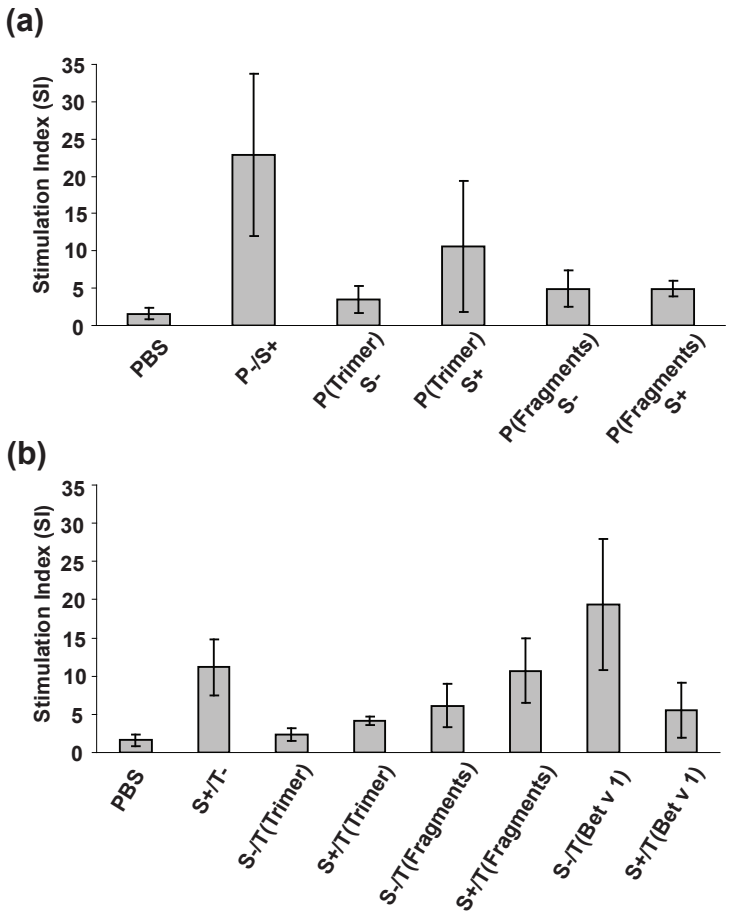
(a)
Stimulation Index (SI)
35
30
25
20
15
10
5
0
PBS
P-/S+
P(Trimer) S-
P(Trimer) S+
P(Fragments) S-
P(Fragments) S+
(b)
Stimulation Index (SI)
35
30
25
20
15
10
5
0
PBS
S+/T-
S-/T(Trimer)
S+/T(Trimer)
S-/T(Fragments)
S+/T(Fragments)
S-/T(Bet v 1)
S+/T(Bet v 1)

Figure S5

Figure Legends:

Figure S1: Measurement of Bet v 1-specific immunoglobulin classes and subclasses by ELISA.

Groups of mice were immunized according to the immunization protocol (Figure 2) and blood samples obtained after the last immunization (day 120) were analyzed for Bet v 1-specific IgM, IgG1, IgG2a, IgG3, and IgA (x-axes) by ELISA as previously described (Linhart B. *et al.*, J. Immunol.2007, 178, 3924-31). O.D. (optical density) values corresponding to the mean Bet v 1-specific antibody levels (+/- SD) measured in **(a)** the prophylaxis groups (P-/S+; P+/S-; P+/S+) and in **(b)** the therapy groups (S+/T-; S-/T+; S+/T+) are shown on the y-axes.

Figure S2: Development of Bet v 1-specific IgE responses in the course of prophaylactic and therapeutic vaccination with Bet v 1-derived peptides

Groups of mice were immunized according to the immunization protocol (Figure 2) and blood samples obtained after each immunization (x-axes: sampling times) were analyzed for Bet v 1-specific IgE responses by ELISA as described (Linhart B. *et al.*, J. Immunol.2007, 178, 3924-31). O.D. (optical density) values corresponding to the mean Bet v 1-specific IgE antibody levels (+/- SD) measured in **(a)** the prophylaxis groups (P-/S+; P+/S-; P+/S+) and in **(b)** the therapy groups (S+/T-; S-/T+; S+/T+) are shown on the y-axes.

Figure S3. Sera from peptide-vaccinated mice inhibit Bet v 1-induced effector cell degranulation *in vitro*.

Sera obtained from different mouse groups (x-axis: PBS; P+/S-; P+/S+; S-/T+; S+/T+) after vaccination (Figure 2, day 120) were loaded on RBL cells, washed and then challenged with Bet v 1 (IS, immune serum). In parallel, RBL cells were loaded with the immune sera, washed and challenged with Bet v1 mixed with the immune serum (Inhibition). Mean percentages +/- SD of ß-hexosaminidase-release are shown on the y-axis for each group.

Figure S4: VPD450-based detection of CD4+T cell proliferation in representative mice from each treatment group by FACS analysis

In a second set of experiments, groups of mice were immunized according to the treatment protocol (Figure 2). In this second set of immunizations an additional group of mice was included which was sensitized and then treated with an irrelevant peptide derived from the unrelated grass pollen allergen Phl p 5 that had been coupled to KLH (S+/irrelevant peptide-KLH). Splenocytes were isolated, labelled with the violet proliferation dye VPD450 (BD Biosciences) according to the manufacturer's instructions and stimulated for 5 days with either Bet v 1, a mixture of the Bet v 1 peptides (Peptides), KLH, or medium alone (see left margin). Subsequently cells were stained with a FITC-labeled anti-mouse CD4 antibody (clone RM4-5) (BD Biosciences) or an isotype control. Cells were analysed on a FACS Canto (Becton & Dickinson, Franklin Lakes, New Jersey, USA). The Figure shows a representative result from one mouse of each group (S+, only sensitized to Bet v 1; S+/irrelevant peptide-KLH, sensitized to Bet v 1, treatment with an irrelevant peptide from Phl p 5 coupled to KLH; S-/peptides KLH, not sensitized but peptide vaccine treated mice; S+/peptides-KLH, sensitized and peptide vaccine treated mice). CD4+ VPD450^{lo} (i.e., proliferating) T cells were gated and percentages of total CD4+ T cells are indicated.

Figure S5. Vaccination with hypoallergenic Bet v 1-derivatives containing T cell epitopes or wildtype Bet v 1 reduces Bet v 1-specific proliferation. Bet v 1-specific splenocyte proliferation of mice which were immunized following the prophylactic **(a)** and therapeutic **(b)** scheme (Figure 2) using either a Bet v 1 trimer, Bet v 1-derived fragments, or wildtype Bet v 1 for prophylaxis and treatment, were measured as described in the methods section. Mean Bet v 1-specific splenocyte stimulation indices +/- SD determined for each mouse group (x-axes) at day 120 are given on the y-axes.

group	IL-2	IL-4	IL-5	IL-10	IFN-γ
PBS	17,9 (± 8,2)	0,5 (± 0,09)	0,2 (± 0,2)	1,5 (± 0,2)	1,9 (± 0,4)
S+/T-	71,3 (± 52,7)	13,2 (± 4,7)	196,4 (± 245,5)	16,8 (± 10,4)	3 (± 2,1)
S-/T+	26,1 (± 9,6)	3 (± 2)	2,2 (± 1,3)	11,4 (± 17,4)	3,7 (± 3,2)
S+/T+	70,6 (± 79,8)	16,2 (± 11,9)	216,2 (± 274)	10,2 (± 8,7)	2,9 (± 1,2)
P-/S+	74,8 (± 42)	8,9 (± 11,2)	296,8 (± 284)	25,6 (± 18,7)	4 (± 2,2)
P+/S-	20,2 (± 3,6)	1,1 (± 0,7)	0,7 (± 0,5)	2,6 (± 1,9)	0,9 (± 0,7)
P+/S+	41,7 (± 16,5)	1,1 (± 0,8)	151,5 (± 114,5)	8,6 (± 5,3)	1,9 (± 1,6)

Table S1. Cytokine response of splenocytes from vaccinated mice in response to Bet v 1.

Splenocytes obtained from different mouse groups were cultivated in the presence of Bet v 1 and cytokine levels (IL-2, IL-4, IL-5, IL-10, IFN-gamma) were measured in the supernatants by Luminex technology (Bio-Plex mouse cytokine assay, Bio-Rad) according to the manufacturer's instructions as previously described (Reginald K, *et al*., J. Allergy Clin. Immunol. 2011, 128, 82-91). Results are shown in pg/ml and SD values for each group are given in parenthesis.