

Nonallergic Individuals Recognize the Same T Cell Epitopes of Bet v 1, the Major Birch Pollen Allergen, as Atopic Patients¹

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The immune response toward allergens in nonallergic healthy individuals was investigated. To boost immune responses, two injections of birch pollen extract were administered to five nonallergic volunteers. T cell lines (TCL) with specificity for Bet v 1, the major birch pollen allergen, were established and analyzed for epitope specificity using overlapping peptides. Forty-nine T cell clones (TCC) specific for Bet v 1 were isolated from TCLs. Comparison with TCL and TCC established from birch pollen-allergic patients was performed. All TCC revealed the Th phenotype. Epitope specificities of TCL and TCC from nonatopics were identical to those found in allergic individuals. No association between MHC class II molecules and particular epitopes could be observed. In nonallergic as well as in allergic individuals, cytokine production in response to specific stimulation revealed a majority of Th-clones producing IL-4 and IFN- γ . However, TCC derived from atopic individuals revealed a higher IL-4/IFN- γ ratio. Immunoblot and ELISA revealed Bet v 1-specific IgG in nonallergic individuals before and after booster injections, but no IgE could be detected. High levels of Bet v 1-specific IgG and IgE could be detected in birch pollen-allergic patients. It can be concluded, that nonatopic and allergic individuals display the same repertoire of T cell specificities. In allergic individuals, the activation of allergen-specific TCC leads to a higher ratio of produced IL-4 vs IFN- γ , which is responsible for enhanced IgE production. *The Journal of Immunology*, 1995, 154: 1932–1940.

In recent years, a pathway of IgE regulation was described for mice and humans (1–3), essentially based on the reciprocal activities of IL-4 and IFN- γ . IL-4 was identified as IgE-switch factor, as was very recently IL-13 (4), and IFN- γ was characterized as the most important antagonist of this effect (5–7). It could be proven that T cell clones (TCC)³ established from atopic individ-

uals, with specificity for allergens or parasite-derived Ags, could characteristically be attributed to the Th2 subset, whereas TCC specific for bacterial Ags produced high levels of IFN- γ and IL-2 upon stimulation, thus belonging to the Th1 subset (8).

In the last few years, several groups have focused on the identification and characterization of allergenic proteins using immunochemical and molecular biologic techniques. cDNA cloning has provided a number of complete deduced amino acid (aa) sequences of important allergens (9–20). The availability of purified recombinant material with known aa sequence made it possible to identify allergen-derived peptides recognized by allergen-specific TCC (13, 21–26). Until now, several major allergens from important sources have been analyzed. It was described that Bet v 1, the major birch pollen allergen (11), and other allergenic proteins harbor multiple T cell epitopes (21–26), which are differentially recognized by distinct patients. Moreover, it could be shown that single individuals harbor multiple T cell specificities for one allergen (24–26).

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³ Abbreviations used in this paper: TCC, T cell clone; TCL, T cell line; SPT, skin prick test; aa, amino acid; SN, supernatant.

Healthy individuals also display an immune response to aero-allergens. Ig production is mainly restricted to IgG production and virtually no allergen-specific IgE Abs can be detected. However, there is only very limited information available as to the nature of the T cell response of nonatopics, with respect to recognized epitopes and cytokine secretion pattern (27–28). Considering this, we concentrated on establishing allergen-specific TCC derived from nonallergic individuals to make a comparison possible with TCC derived from allergic individuals. In this study, using our model allergen Bet v 1, we compare Ab and cellular immune responses of nonallergic and allergic individuals applying serologic techniques (immunoblotting) and cellular systems (allergen-specific T cell lines and clones).

Materials and Methods

Study design

This study includes five healthy, nonallergic individuals and five patients with atopic disease. The study was reviewed and approved by the Ethics Commission of the University of Vienna, Austria. Nonallergic individuals included five healthy volunteers (two female, three male). HLA-typing (DRB, DQB) was performed as described (29) and revealed for individual BP: DRB1*16, DRB1*11; DRB3, DRB5; DQB1*01, DQB1*03; for individual CE: DRB1*15, DRB1*04; DRB4, DRB5; DQB1*01, DQB1*03; for individual RS: DRB1*10, DRB1*11; DRB3; DQB1*01, DQB1*03; for individual SS: DRB1*01, DRB1*07; DRB4; DQB1*01, DQB1*02; for individual ZS: DRB1*12, DRB1*13; DRB3; DQB1*03, DQB1*03. Neither of these individuals ever experienced symptoms that could be connected with type I allergy. Skin-prick tests (SPT) using a panel of selected allergens (including tree pollens, grass pollens, house dust mite, and animal dander; Soluprick, ALK, Horsholm, Denmark), IgE measurements, and cellular proliferation assays (described below) were performed to prove the nonallergic state of the probands. Injections: the specific immune response of the probands toward Bet v 1 was boosted to render possible the establishment of allergen-specific T cell lines (TCL) and TCC from PBMC. For this reason, individuals received two injections of an Al(OH)₃-adsorbed birch pollen extract. This vaccine (ALK SQ 100; ALK) is routinely used for hypsensitization (specific immunotherapy) of birch pollen allergic patients. Injections were administered subcutaneously in a 2-wk interval, doses of 1000 and 5000 SQ-Units (ALK) were used. Before each injection, SPT and serum-IgE evaluation were performed to exclude the presence of allergen-specific IgE Abs. Blood was collected before the first injection and 2 wk after the last injection. Birch pollen-allergic volunteers: Five birch pollen-allergic patients (one female, four male), as proved by typical case history, positive skin test, and displaying IgE-binding to Bet v 1, the major birch pollen allergen, were used for comparison. HLA typing revealed the following MHC class II types: GZ: DRB1*07, DRB1*08; DRB4; DQB1*03, DQB1*04; MS: DRB1*04, DRB1*13; DRB3, DRB4; DQB1*06, DQB1*03; TF: DRB1*15, DRB1*07; DRB4, DRB5; DQB1*01, DQB1*02; WD: DRB1*15, DRB1*07; DRB4, DRB5; DQB1*01, DQB1*02; WF: DRB1*14, DRB1*11; DRB3; DQB1*01, DQB1*03.

Allergen preparations

Natural Bet v 1 was purified from aqueous pollen extracts (*Betula verrucosa*, Allergon, Engelholm, Sweden) by C₈ RP-HPLC (Hypersil WP 300 10 μ m, 8 $\times$ 250 mm) performing a linear gradient of 2-propanol within 50 min at room temperature (solvent A: H₂O/0.1% TFA, B: 90% 2-propanol/0.1% TFA; gradient: 0–80% B; flow rate 1.8 ml/min). UV absorbance was monitored at 280 nm. Aliquots were analyzed by SDS-PAGE and immunoblot using mAbs and patients' sera (30, 31). Expression and purification of recombinant nonfusion Bet v 1: the recombinant non-fusion protein was expressed by using the pKK 223–3 expression vector (32; Pharmacia, Uppsala, Sweden), the promoter of which is reg-

ulated by the *lac* repressor and can be induced by adding isopropyl-beta-D-thiogalactoside (IPTG) to the medium as described (33). Concentrations: The allergen concentrations applied in proliferation assays were 5 μ g/ml (1 μ g/well) for natural and recombinant Bet v 1. Protein concentrations of purified protein samples were determined by the micro-Kjeldahl method using glycine as standard (34).

Immunoblots

Immunoblots were performed as described (35). Sera of the probands were incubated in 1:4 (IgE) and 1:10 (IgG) dilution with nitrocellulose strips coated with nBet v 1. Bound IgE was detected using a radiolabeled polyclonal rabbit anti-human IgE Ab (Pharmacia). IgG immunoblots were performed using a polyclonal rabbit anti-human IgG Ab (Jackson/Dianova, Hamburg, Germany), which was detected by a radiolabeled donkey anti-rabbit Ab (Amersham, Amersham, U.K.).

IgE-, IgG-, ELISA

An ELISA system was used to investigate Bet v 1 specific IgE and IgG titers of our probands. Microtiter plates (M129A, Dynatech MT, Zug, Switzerland) were coated with the monoclonal anti-Bet v 1 Ab BIP 1 as capture Ab as described (30, 36). Tests were performed using rBet v 1 (33).

Proliferation assays

10⁵ cells (PBMC) were cultured in triplicates in 96-well plates (200 μ l) in the presence of 20, 10, and 5 μ g Bet v 1 per ml, for 6 days at 37°C, 5% CO₂, humidified atmosphere. In the last 16 h of culture [³H]TdR (Amersham; 0.5 μ Ci per well) was added and the incorporated radioactivity was measured by scintillation counting.

Allergen-specific T cell lines

Allergen-specific TCL were obtained by a technique described (8, 25). Briefly, 0.5 $\times$ 10⁶ PBMC were stimulated with individual optimal concentrations of purified natural Bet v 1 (5–20 μ g/ml) in Ultra Culture Medium (Bio Whittaker, Walkersville, MD) supplemented with 2 mM/l glutamine and 2 $\times$ 10^{–5} M 2-ME in 24-well flat-bottom culture plates (3524 Costar, Cambridge, MA) for 5 days. Subsequently, human r-IL-2 (10 U/ml, Boehringer-Mannheim, Mannheim, Germany) was added and the culture continued for an additional 7 days. Viable T cell blasts were then separated by Ficoll/Hypaque (Pharmacia), and Bet v 1 specificity was assessed by an [³H]TdR incorporation assay. TCL were tested with nBet v 1, rBet v 1, and overlapping peptides representing the amino acid sequence of Bet v 1 to reveal the repertoire of T cell recognition.

Allergen-specific T cell clones

T cell blasts from Bet v 1-specific TCL were seeded in limiting dilution (0.3 cells/well) in 96-well round-bottom plates (Nuncclone, Nunc Corp., Roskilde, Denmark) in the presence of 10⁵ irradiated (5000 rad) allogeneic PBMC as “feeder cells”, 1% PHA v/v (Life Technologies, Grand Island, NY) and rIL-2 (4 U/well) in the above-described medium. Growing microcultures were then expanded at weekly intervals with fresh feeder cells and rIL-2. The specificity of TCC was assessed as previously described (25). Briefly, 2 $\times$ 10⁴ T cell blasts were incubated in triplicate cultures in 96-well plates in the presence of 10⁵ autologous irradiated PBMC, with whole birch pollen extract, purified nBet v 1, rBet v 1, and overlapping Bet v 1-peptides. Microtiter plates were then incubated for 48 h at 37°C, 5% CO₂-humidified atmosphere. After pulsing for 16 h with [³H]TdR, cells were harvested, and the incorporated radioactivity was measured by scintillation counting. When the stimulation index (ratio between cpm obtained in cultures with TCC plus autologous irradiated PBMC plus Ag and cpm obtained in cultures containing TCC and PBMC alone) was greater than 10, responses were considered as positive.

Analysis of the phenotype of TCC

Abs: FITC-labeled mAb anti-Leu 4/CD3, anti-Leu 3a/CD4, anti-Leu 2a/CD8, anti-T cell Ag Receptor TCR-1 α/β WT31 and anti-TCR γ/δ 1 (Becton Dickinson, Mountain View, CA) were used to analyze the phenotype of the TCC. Flow cytometry analysis: TCC and irradiated feeder

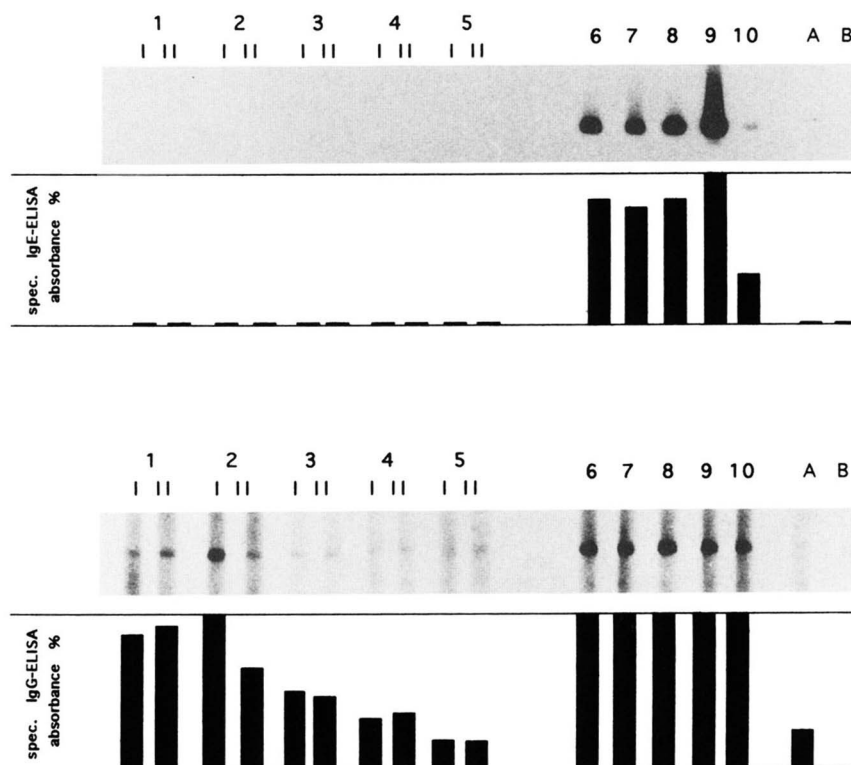


FIGURE 1. IgE-immunoblot: serum-IgE reactivity of five nonallergic donors (1–5: BP, CE, RS, SS, ZS) to recombinant Bet v 1. *I*, IgE-binding to rBet v 1 before, and *II*, after administration of two injections of birch pollen extract (6–10: birch pollen-allergic individuals: GZ, MS, TF, WD, WF). Controls: A, serum pool of 13 nonallergic donors; B, buffer. Bars below the bands symbolize results of Bet v 1-specific IgE-ELISA (% absorbance). IgG-immunoblot: serum-IgG reactivity of five nonallergic donors (1–5: BP, CE, RS, SS, ZS) to recombinant Bet v 1. *I*, IgG-binding to rBet v 1 before, and *II*, after administration of two injections of birch pollen extract (6–10: birch pollen-allergic individuals: GZ, MS, TF, WD, WF). Controls: A, serum pool of 13 non-allergic donors; B, buffer. Bars below the bands symbolize results of Bet v 1-specific IgG-ELISA (% absorbance).

cells alone were harvested, washed, and 10^5 cells were incubated for 30 min on ice with the appropriate dilution of mAb or isotype-matched control Abs. After washing three times with PBS containing 0.3% BSA and 0.3% NaN_3 , the cells were stained with propidium bromide to allow exclusion of dead cells, and analyzed on a FACScan (Becton Dickinson).

Overlapping peptides

A panel of 75 peptides was synthesized according to the Bet v 1 sequence by Cambridge Research Biochemicals Limited, Cambridge, U.K. (37). The dodecapeptides were overlapping for two amino acids (neighbors shared ten residues). For our proliferation assays, a concentration of 1 $\mu\text{g}/\text{well}$ (5 $\mu\text{g}/\text{ml}$) was used.

Specific stimulation of TCC for cytokine production

10^6 clone cells were washed and incubated with 10^6 irradiated autologous APC in the presence of Bet v 1 (10 $\mu\text{g}/\text{ml}$). Supernatants (SN) from the allergen-stimulated cultures were collected after 24 h of incubation.

Cytokine assays

IL-4 levels in the SN were measured using a sandwich ELISA technique (Quantikine; R&D Systems, Minneapolis, MN), sensitivity threshold: 3 pg/ml. SN with IL-4 contents of >3000 pg/ml were retested in 1:2 dilution. IL-2, IFN- γ were determined by solid-phase ELISA (Medgenix Diagnostix, Fleurus, The Netherlands). IL-10 was measured using a solid-phase sandwich ELISA assay obtained from Biosource International, Camarillo, CA. Recombinant and natural cytokine standards were used as

references. SN collected from cultures containing allergen and irradiated feeder cells alone were used as controls.

Results

Skin prick test

SPT using a panel of selected allergens (including tree pollens, grass pollens, house dust mite and animal dander) revealed nonreactivity in 5/5 nonallergic patients. In the atopic patients' group all individuals revealed positive skin reactivity when tested with birch pollen extract.

Immunoblots

IgE immunoblots, performed using sera collected before booster injections were administered, revealed no IgE-binding to Bet v 1 in nonallergic subjects. The healthy volunteers received two injections of birch pollen extract. This led in no case to induction of Bet v 1-specific IgE. All five probands recognized Bet v 1 with their IgG Abs. No increase of IgG or induction of IgE could be observed after two injections of birch pollen extract (Fig. 1). The allergic individuals revealed IgE and IgG reactivity to birch pollen

proteins in immunoblots. All atopic patients displayed IgE-binding to Bet v 1 (Fig. 1).

IgE-, IgG- ELISA

In the nonallergic collective, ELISA measurements of Bet v 1-specific IgE in the serum were negative and remained negative after administration of birch pollen extract (Fig. 1). Bet v 1-specific IgG was detectable. IgE and IgG titers revealed no significant increase between the blood sample collected before the first injection and the probe collected 2 wk after the second injection (Fig. 1). Birch pollen-allergic subjects displayed IgE- and IgG-binding to rBet v 1. Bet v 1-specific IgG titers were generally higher in birch pollen-allergic patients than in healthy individuals (Fig. 1).

Proliferation assays

Two weeks after the second s.c. injection of birch pollen extract, proliferation of PBMC could be observed in a 6-day allergen-stimulation assay in every individual.

T cell lines

After two s.c. injections of birch pollen extract, it was in every case possible to establish Bet v 1-specific TCL from the nonallergic volunteer collective. The TCL reacted with purified natural as well as with purified recombinant Bet v 1. Reactivity with the natural Bet v 1 preparation was in most cases more pronounced.

T cell clones

In the case of nonatopics, it was possible to isolate 2–15 TCC specific for Bet v 1 per proband. Cloning efficiency for Bet v 1-specific TCC was calculated and revealed yields from 0.3 to 2.5% depending on specificity of the TCL. Forty-nine Bet v 1-specific TCC of the healthy probands reacted with purified natural Bet v 1; however, as described previously (26, 33, 38), some of the TCC did not proliferate in response to recombinant Bet v 1. In the case of allergic patients, cloning efficiency for Bet v 1-specific TCC reached 3 to 20%. Thirty-three TCC of allergic patients reacting with natural and with recombinant Bet v 1 were included in this study.

Epitope specificity of T cell lines and clones

TCLs from all donors were tested with overlapping peptides in proliferation assays. Moreover, sixteen TCC from nonallergic donors and 33 TCC from birch pollen-allergic volunteers, displaying strong reactivity with rBet v 1, were epitope mapped. In nonatopic individuals, epitope mapping using overlapping peptides revealed T cell recognition of 1 (donor SS; Table I) to 6 (donors CE, ZS; Table I) epitopes per individual. In most cases, three neighboring peptides induced proliferation. Four of the five probands recognized peptides representing Bet v 1-aa 109–128

(DGGSLKISNKYHTKGDHEV); three of five individuals reacted with the sequence ETLRAVESYLLAHS, Bet v 1: aa-position 141–156, with the sequence aa 97–112 (KISNEIKIVATPDGGS) and DHTNFKYNYSVIEGGP (aa 75–90). The sequence part Bet v 1-aa 63–80 (PFKYVK DRVDEVDHTNFK) represented a T cell epitope for two nonallergic donors. Five other T cell-stimulating sequences could be found once (Bet v 1-aa 1–16; 7–22; 25–40; 33–48; 51–64, and 125–140). In atopic patients, T cells from four of five patients recognized the sequence DHTNFKYNYSVIEGGPIG (aa 75–92). Three of the five reacted with KISNEIKIVATPDGGS (aa 97–112) and ETLRAVESYLLAHS (aa 141–156). Two of five recognized GSKISNKYHTKGDHEV (aa 111–128), VAPQAISVENIEGNG (aa 33–48) and peptides representing the region: aa 63–80 (PFKYVKDRVDEVDHTNFK) and aa 7–24 (TETTSVIPAARLFKAFIL). Four other epitopes could be observed once. Epitope mapping data are summarized in Figure 2.

Cytokine production pattern

In nonatopic individuals, 9/49 (18.4%) TCC displayed a high IL-4 vs low IFN- γ production (Th-2-like pattern of cytokine production); 11/49 (22.4%) revealed high IFN- γ vs low IL-4 synthesis (Th 1-like type of cytokine production). In TCC from atopic patients, 13/33 (39.4%) TCC displayed a high IL-4 vs low IFN- γ production (Th-2-like pattern of cytokine production), 1/33 (3%) revealed a high IFN- γ synthesis vs low IL-4 production (Th 1-like type of cytokine production). 29/49 (59.2%) TCC from nonallergic donors and 19/33 (57.6%) TCC from allergic individuals produced considerable amounts of IL-4 as well as of IFN- γ in response to stimulation, and can thus be designated Th 0 clones. However, also in these TCC a tendency toward high IL-4/IFN- γ ratios was obvious in the atopic group (Fig. 3). In the case of TCC from nonallergic donors, IL-10 could be detected in 40 of 48 (80%) SN tested, in case of SN of TCC of allergic donors in 66% (Table I). IL-2 contents of SN were generally low, obviously reflecting utilization during the proliferation of TCC. In the controls, no cytokine production could be measured in 24-h SN of nonstimulated cultures or irradiated APC. Cytokine production of allergen-specific TCC from nonallergic and allergic donors in response to specific (Bet v 1) stimulation is summarized in Table I. Figure 3 shows IL-4 and IFN- γ production of Bet v 1-specific TCC from allergic and nonallergic donors. In Table II, TCC reacting with the most frequently recognized epitopes (Bet v 1-aa 75–92, Bet v 1-aa 109–128, Bet v 1-aa 141–156) are directly compared.

Discussion

In recent years, the crucial role of the Th in the induction and maintenance of Type I allergic diseases has been realized. A dichotomy of Th functions could be demonstrated for mice (1, 6) and confirmed in humans (2, 3). Ab

Table 1. Cytokine production by human Bet v 1-specific T cell clones^a in response to Ag-specific stimulation

Nonallergic Individuals						Allergic Patients					
TCC	Epitope Bet v 1	IL-2 ^a (U/ml)	IFN-γ ^a (pg/ml)	IL-4 ^a (pg/ml)	IL-10 ^a (pg/ml)	TCC	Epitope Bet v 1	IL-2 ^a (U/ml)	IFN-γ ^a (pg/ml)	IL-4 ^a (pg/ml)	IL-10 ^a (pg/ml)
BP13	75-90	1.5	450	<3	>1500	GZ3	127-140	<0.1	80	52	<5
BP38	75-90	<0.1	150	3000	1000	GZ11	77-92	<0.1	80	1100	835
BP53	75-90	9	105	45	>1500	GZ12	113-128	<0.1	90	940	<5
BP60	141-156	<0.1	50	250	160	GZ15	113-128	<0.1	100	840	299
BP69		<0.1	80	122	440	GZ16	113-128	<0.1	500	3300	>1500
BP91	111-126	<0.1	150	372	1000	GZ20	113-128	<0.1	550	5360	1454
BP95		<0.1	80	<3	182	GZ24	113-128	<0.1	480	3072	338
BP102		5	400	3210	>1500	GZ25	77-92	<0.1	155	2524	470
BP150		0.8	75	2000	228	GZ29	113-128	<0.1	>1500	2936	>1500
BP173	75-90	<0.1	125	1100	846	GZ11II	77-92	<0.1	100	160	<5
BP181	75-90	3.4	100	134	960	GZ17II	77-92	<0.1	80	1680	<5
CE24	141-156	<0.1	890	94	ND	GZ21II	77-92	<0.1	80	>6000	<5
CE43	111-126	35	1400	1304	498	GZ29II	61-76	<0.1	>1500	1384	>1500
CE50		1.4	1000	30	57	MS2	65-80	<0.1	250	<3	<5
CE57		5.6	600	1392	1278	MS5	141-156	<0.1	85	420	410
CE58		1.2	1350	442	657	TF1II	95-110	1.2	90	82	12
CE90		1.8	1100	67	54	TF7II	11-26	<0.1	1580	2600	238
CE105		8	1400	>6000	1170	TF10II	141-156	2.4	50	3000	<5
CE116	65-80	36	1350	729	1554	TF23II	97-112	<0.1	<1.5	48	<5
CE120		29	1350	312	1191	TF23III	97-112	<0.1	65	40	<5
CE126		11	1350	14	990	WD18	1-16	<0.1	<1.5	666	764
CE132	111-126	4.5	1300	118	465	WD22	1-16	<0.1	190	886	569
CE139		3	1400	878	351	WD24	141-156	>30	>1500	>6000	>1500
CE148		<0.1	1450	5	1092	WD25	141-156	<0.1	330	>6000	934
CE170		1.3	500	4	51	WD27	1-16	<0.1	270	1426	<5
CE199		1.3	1200	68	1140	WF3	111-126	<0.1	1800	2410	<5
RS17		<0.1	70	<3	816	WF24	111-126	2.6	430	1436	14
RS31	141-156	<0.1	65	220	<5	WF29	75-90	<0.1	130	1442	>1500
SS8		<0.1	175	<3	<5	WF41	111-126	<0.1	100	2362	70
SS14		<0.1	1500	36	276	WF42	111-126	<0.1	585	134	50
SS44		>30	1480	18	ND	WF14II	75-90	5.7	215	3404	594
SS31		1.2	100	670	24	WF31II	75-90	<0.1	185	3048	716
SS53		<0.1	65	<3	670	WF72II	75-90	<0.1	560	2828	6
SS63		<0.1	80	182	1290						
SS78		<0.1	100	153	210						
ZS5		<0.1	70	1002	354						
ZS12		<0.1	60	3150	180						
ZS27		<0.1	55	1200	200						
ZS36	75-90	2.7	100	900	<5						
ZS53		2	150	3000	300						
ZS85		<0.1	55	<3	<5						
ZS93	63-78	19	70	67	<5						
ZS101		<0.1	65	1200	<5						
ZS114		<0.1	650	1100	<5						
ZS118		<0.1	55	2000	<5						
ZS138		<0.1	60	1000	<5						
ZS142		<0.1	85	376	40						
ZS150		<0.1	130	77	40						
ZS167	75-90	19	360	3000	1000						

^a One million T cells were stimulated with 10 μg Bet v 1 in 1 ml medium; cytokines were measured in 24-h supernatants by specific ELISA.

FIGURE 2. Bet v 1 T cell epitopes recognized by Bet v 1-specific T cell lines and T cell clones established from five nonallergic and five birch pollen-allergic individuals. Nonallergic volunteers: BP: DRB1*16, DRB1*11; DRB3, DRB5; DQB1*01, DQB1*03; CE: DRB1*15, DRB1*04; DRB4, DRB5; DQB1*01, DQB1*03; RS: DRB1*10, DRB1*11; DRB3; DQB1*01, DQB1*03; SS: DRB1*01, DRB1*07; DRB4; DQB1*01, DQB1*02; ZS: DRB1*12, DRB1*13; DRB3; DQB1*03, DQB1*03; Allergic individuals: GZ: DRB1*07, DRB1*08; DRB4; DQB1*03, DQB1*04; MS: DRB1*04, DRB1*13; DRB3, DRB4; DQB1*06, DQB1*03; TF: DRB1*15, DRB1*07; DRB4, DRB5; DQB1*01, DQB1*02; WD: DRB1*15, DRB1*07; DRB4, DRB5; DQB1*01, DQB1*02; WF: DRB1*14, DRB1*11; DRB3; DQB1*01, DQB1*03.

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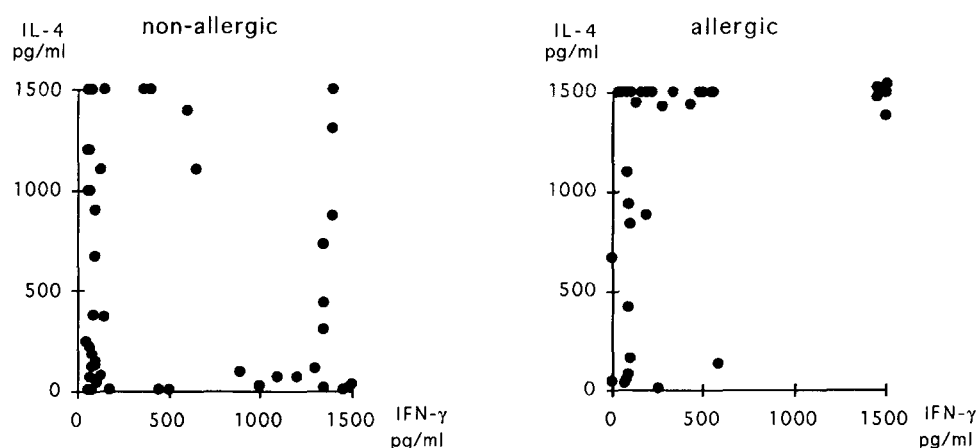


FIGURE 3. IL-4 vs IFN- γ production in 49 Bet v 1-specific T cell clones established from the peripheral blood of nonallergic individuals and 33 Bet v 1-specific T cell clones from birch pollen-allergic individuals.

Table II. Direct comparison of TCC established from nonallergic and allergic individuals^a

Bet v 1 Peptides	TCC	Nonallergic			TCC	Allergic		
		IL-4 pg/ml	IFN- γ pg/ml	IL-4/IFN- γ		IL-4 pg/ml	IFN- γ pg/ml	IL-4/IFN- γ
⁷⁵ DHTNFKYNSVI/EG/GP/IG ⁹²	BP13	<3	450	<0.01	GZ11	1100	80	13.8
	BP38	3000	150	20	GZ17II	1680	80	21
	BP53	45	105	0.4	GZ21II	>6000	80	>75
	BP173	1100	125	8.8	WF14II	3404	215	15.8
	BP181	134	100	1.3	WF72II	2828	560	5
	ZS36	900	100	9				
	ZS167	3000	360	8.3				
¹¹¹ GSILKISNKYHT/KG/DH ¹²⁶					GZ20	5360	550	9.7
	BP91	372	150	2.5	GZ24	3072	480	6.3
	CE43	1304	1400	0.9	GZ29	2936	>1500	ND
	CE132	118	1300	0.1	WF3	2140	1800	1.3
					WF24	1436	430	3.3
					WF41	2362	100	23.6
¹⁴¹ ETLLRAVESYLL/AH/SD ¹⁵⁶	BP60	250	50	5	MS5	420	85	4.9
	CE24	94	890	0.1	WD24	>6000	>1500	ND
	RS31	220	65	3.9	WD25	>6000	330	>20
					TF10II	3000	50	60

^a TCC are specific for three dominant epitopes on the Bet v 1 allergen.

synthesis is regulated by a reciprocal interplay of Th1 cells, which produce high amounts of IFN- γ and IL-2 and Th2 cells, which produce high amounts of IL-4, the strongest inducer of IgE synthesis. Bacterial and viral Ags seem preferentially to induce the Th1 pathway, whereas some parasitic Ags and atopic allergens mainly expand Th2 cells, consequently leading to the production of specific IgE. Knowledge of the molecular structures (i.e., aa-sequence) of important allergens (9–20) made the analysis of epitopes on these proteins possible (21–26). In several studies, using different techniques, it could be shown that allergens harbor multiple T cell epitopes (24–26). However, the limited number of reports did not rule out the existence of disease-related epitopes, possibly associated with particular MHC class II haplotypes (29, 39–40). The production of allergen-spe-

cific TCL and TCC derived from nonallergic individuals is a difficult procedure, because the cellular and Ab responses toward allergens are weak in nonatopics (Fig. 1; 28). This explains the limited information about the characteristics of this response (2, 27). However, the presence of specific IgG in sera of nonallergic individuals clearly indicates a recognition of allergenic proteins by the immune system (Fig. 1). By boosting the specific immune response to Bet v 1, the major birch pollen allergen, with two injections of birch pollen extract, we achieved cellular responses to natural and recombinant Bet v 1 which made possible the establishment of specific TCL and TCC from the peripheral blood of nonallergic volunteers. TCL were established with natural Bet v 1, a mixture of different allergenic isoforms. Not all TCC reacted with our recombinant

protein, which represents only one possible isoform sequence (33, 38). In the case of TCC from allergic individuals, produced using the same culture system, the same phenomenon was evident; however, larger numbers of TCC were available, and only rBet v 1-reactive TCC were included in the study. The cloning efficiency of allergen-specific TCC was significantly lower in non-allergic as compared with allergic individuals. Moreover, in accordance with the weak cellular responses to allergens in nonatopic individuals, the expansion of established TCC to high cell numbers was not always feasible. Therefore, a selection of TCC and T cell specificities cannot be excluded in our in vitro system. Epitope mapping was performed using TCL. Specificity of isolated TCC proved the epitopes found in the respective lines. We provide evidence that healthy individuals with different genetic background recognize the same T cell epitopes as allergic patients. All important T cell epitopes mapped with TCL and TCC from allergic individuals could also be detected in non-allergics (Fig. 2). Consistent with these results, we found no evidence for association between recognition of particular T cell epitopes and expression of special HLA class II molecules (29, 39–40).

It could be demonstrated that the majority of allergen-specific TCC derived from allergic individuals display a Th2-like pattern of cytokine production (2, 3). It is of particular interest to compare these results with data obtained with allergen-specific TCC from healthy, nonatopic individuals. As expected, looking at the total of Bet v 1-specific TCC investigated, we found a tendency toward high IL-4/IFN- γ ratios in TCC from atopic individuals; this ratio was significantly lower in TCC derived from nonallergic individuals (Table I, Fig. 3). When comparing TCC isolated from nonallergic donors with TCC from atopic volunteers recognizing the same epitopes on Bet v 1, high IL-4, rather than low IFN- γ levels, seemed to be responsible for this result (Table II). Within the nonallergic collective, some individuals seemed to be more biased toward Th1 (individual CE, Table I) others to Th2, (individual ZS, Table I). IL-10, a cytokine described to be characteristic for Th2 cells in mice (41), could be found in almost every TCC-SN, irrespective of the Th subset type (42; Table I).

Based on our findings, we conclude that individuals without any evidence of atopic diseases display T cell reactivity toward a limited number of T cell epitopes of allergenic proteins. These peptides lead to activation of different Th subtypes. In case of nonallergic, in contrast to allergic individuals, T cells respond to specific stimulation with a balanced production of agonist and antagonist (IL-4/IFN- γ). This balance is obviously a prerequisite for a controlled humoral immune response to "innocent" (i.e., nonpathogenic) ubiquitous Ags, such as pollen proteins. This situation subsequently leads to synthesis of Ig of the IgG isotype and prevents IgE production. Therapeutic concepts (such as specific immunotherapy; 43) should there-

fore focus on causal therapies directed against the impaired cytokine balance of allergen-specific T lymphocytes in atopic individuals. It is conceivable that immunotherapy using peptides representing dominant T cell epitopes could be capable of modulating the T cell response of allergic patients (44–46) and therefore prevent the production of IgE Abs at the earliest stage.

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