

T CELL CLONES SPECIFIC FOR *Bet v* I, THE MAJOR BIRCH POLLEN ALLERGEN, CROSSREACT WITH THE MAJOR ALLERGENS OF HAZEL, *Cor a* I, and ALDER, *Aln g* I

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Abstract—Tree pollens are responsible for type I allergies during the flowering season in spring. Pollens from birch, hazel and alder constitute the most important allergen sources in this respect in the northern hemisphere. Human IgE antibodies, specific for the major allergens of these pollens, are known to crossreact, and in general every tree pollen allergic patient is sensitized to these three pollen allergens. In this study we investigated eight T-helper cell clones (CD3+, CD4+, TCR α/β) with specificity for *Bet v* I, the major birch pollen allergen, as proved by reactivity with purified natural as well as with recombinant allergen. The T cell clones were used to investigate common T cell epitopes of the *Bet v* I molecule with *Cor a* I, the major allergen of hazel pollen and *Aln g* I, the major allergen of alder pollen. All eight T cell clones reacted with all three proteins with different intensity. Moreover, three T cell clones, which were known to react with immunodominant T cell epitopes on the *Bet v* I molecule, were tested for reactivity with dodecapeptides synthesized according to the corresponding homologous regions of the *Cor a* I and *Aln g* I sequence. All the peptides induced strong T cell proliferation, indicating the existence of multiple cross-reacting epitopes. These findings will have an impact on the production of vaccines for immunotherapy of tree pollen allergies.

INTRODUCTION

Type I allergy to tree pollens is a common disease in Northern and Central Europe as well as in certain areas of Northern America. Pollens from birch (*Betula verrucosa*), hazel (*Corylus avellana*) and alder (*Alnus glutinosa*), all of the order of *Fagales*, represent three important allergen sources which cause tree pollen allergy (Ipsen *et al.*, 1985; Jarolim *et al.*, 1989a). Previous studies showed that 95% of all tree pollen allergic patients display IgE-binding to *Bet v* I, the major allergen of birch pollen (BP), and that 60% react exclusively with this protein, indicating the importance of this protein in tree pollen allergy (Jarolim *et al.*, 1989a). Characterization of the major tree pollen allergens *Bet v* I (birch), *Cor a* I (hazel) and *Aln g* I (alder) by 2D PAGE (two-dimensional polyacrylamide gel electrophoresis) and inhibition studies showed extensive cross-reactivity of patients' IgE (Rohac *et al.*, 1991). Furthermore,

monoclonal antibodies raised against *Bet v* I bound strongly to related proteins in hazel and alder pollen extracts (Rohac *et al.*, 1991), and cross-hybridization studies using a *Bet v* I encoding cDNA (Breiteneder *et al.*, 1989) as a probe revealed high homology between *Bet v* I, *Cor a* I and *Aln g* I at the nucleic acid level (Valenta *et al.*, 1991a) and between the deduced amino acid sequences (Ipsen and Hansen, 1991). In spite of the fact that the cross-reactivity of patients' IgE among the major allergens of different pollens of the order *Fagales*, leading to cross-allergy, is very well documented (Ipsen *et al.*, 1985; Ipsen and Hansen, 1991; Nüchel-Petersen *et al.*, 1988; Valenta *et al.*, 1991a), nothing is known about putative cross-reactive T cell epitopes.

In this study, we investigated whether the major allergens *Bet v* I, *Cor a* I and *Aln g* I share T cell epitopes. For this purpose, *Bet v* I-specific T cell clones (TCC) were tested for reactivity with the purified major allergens of birch, hazel and alder pollens. In three cases we also investigated the TCC proliferation with dodecapeptides representing immunodominant T cell epitopes of *Bet v* I (Ebner *et al.*, 1993a, b), and corresponding amino acid sequence regions of the *Cor a* I (Breiteneder *et al.*, 1993) and *Aln g* I (Breiteneder *et al.*, 1992) molecule.

MATERIALS AND METHODS

Patients

Peripheral blood was collected from seven tree pollen-allergic patients. Type I allergy to BP was proved by

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Abbreviations: TCC, T cell clone; BP, birch pollen; n-*Bet v* I, natural *Bet v* I; r-*Bet v* I, recombinant *Bet v* I; SDS-PAGE, sodium dodecyl sulphate-polyacrylamide gel electrophoresis; cDNA, complementary deoxyribonucleic acid; PBMC, peripheral blood mononuclear cells; PBS, phosphate buffered saline; r-IL-2, recombinant IL-2; TCR, T cell receptor.

typical case history, positive skin tests and positive RAST (classes 2.8–5). Moreover, IgE-binding to purified natural *Bet v* I (n-*Bet v* I) as well as to purified recombinant *Bet v* I (r-*Bet v* I) and to the purified major allergens of hazel (*Cor a* I) and alder (*Aln g* I) was investigated by immunoblot experiments. Peripheral blood mononuclear cells (PBMC) were used for cell culture experiments. All patients' PBMC displayed positive lymphocyte proliferation in response to stimulation with extracts from birch pollen, hazel pollen, alder pollen and the respective purified major allergens.

Allergen protein preparations

n-*Bet v* I was purified from aqueous extracts (Jarolim *et al.*, 1989a) of pollen (*Betula verrucosa*, Allergon AB, Engelhom, Sweden) by affinity chromatography using the immobilized monoclonal antibody BIP 1 as described (Jarolim *et al.*, 1989b). The eluted material was dialysed against phosphate buffered saline (PBS), precipitated by addition of a four-fold volume of acetone and centrifuged (Minifuge RF, Hereus; 5000 rpm). The pellet was dried in a desiccator, dissolved in 5 M urea, extensively dialysed against PBS, and after sterile filtration used for cell culture.

Expression and purification of recombinant non-fusion Bet v I. The recombinant non-fusion protein was expressed in *Escherichia coli* by using the pKK 223-3 expression plasmid (Amann *et al.*, 1983; Pharmacia, Uppsala, Sweden) and purified as described (Ebner *et al.*, 1993a; Ferreira *et al.*, 1992).

Purification of Cor a I and *Aln g* I. Aqueous extracts of pollens from hazel and alder were prepared as previously described (Jarolim *et al.*, 1989a) and loaded onto a preparative reversed-phase high-performance liquid chromatography column. *Cor a* I and *Aln g* I were subsequently eluted employing a linear gradient within 60 min at room temperature.

The protein concentration of all purified samples was determined by the micro-Kjeldahl method using glycine as standard (Jacobs, 1959). Aliquots were analysed by SDS-PAGE and subsequent silver staining (Merril *et al.*, 1981) and immunoblot.

Peptides

A panel of peptides was synthesized by Cambridge Research Biochemicals (Cambridge, U.K.; Maeji *et al.*, 1990). The clones BE15, DF15 and HC5 were tested in proliferation assays as described by Ebner *et al.* (1993a). The concentration of peptides was 1 µg/well (5 µg/ml). The following T cell clones were tested with the peptides shown (sequences in the international one-letter code for amino acids)

BE15:	
LRAVESYLLAHS	(<i>Bet v</i> I; amino acids 144–155)
LRAVETYLLAHS	(<i>Cor a</i> I; amino acids 144–155)
LKAVESYLLAHS	(<i>Aln g</i> I; amino acids 144–155)

DF16:

PFKYVKDRVDEV	(<i>Bet v</i> I; amino acids 63–74)
RYKYVKERVDEV	(<i>Cor a</i> I; amino acids 63–74)
PFKYVKERVDEV	(<i>Aln g</i> I; amino acids 63–74)

HC5:

TNFKYNYSVIEG	(<i>Bet v</i> I; amino acids 77–88)
TNFKYSYTVIEG	(<i>Cor a</i> I; amino acids 77–88)
VNFKYSFSVIEG	(<i>Aln g</i> I; amino acids 77–88)

IgE immunoblots

IgE-immunoblots were performed as described (Jarolim *et al.*, 1989a). Sera of the seven patients were incubated in a 1:4 dilution with nitrocellulose strips coated with n-*Bet v* I, r-*Bet v* I, *Cor a* I and *Aln g* I, respectively. Bound IgE was detected using a radio-labelled polyclonal rabbit anti-human IgE antibody (Pharmacia).

Allergen-specific TCC

Allergen-specific TCC were obtained by a technique described elsewhere (Parronchi *et al.*, 1991). Briefly, 0.5×10^6 PBMC were stimulated with individual optimal n-*Bet v* I concentrations (5–20 µg/ml), determined in a proliferation assay by titration in Aim V medium (Gibco, Grand Island, NY) supplemented with 2 mM/l glutamine and 2×10^{-5} M 2-ME in 24-well flat-bottomed culture plates (3524 Costar, Cambridge, MA) for five days. Then, human recombinant IL-2 (r-IL-2; 20 U/ml, Genzyme, Cambridge, MA) was added and the culture continued for an additional seven days. Viable T cell blasts were then separated by Ficoll Hypaque (Pharmacia), and *Bet v* I specificity was assessed by a ^3H -thymidine (Amersham International, U.K.) incorporation assay. Thereafter, T cell blasts were seeded in limiting dilution (0.3 cells/well) in 96-well round-bottomed plates (Nuncclone, Nunc, Denmark) in the presence of 10^5 irradiated (5000 rad) allogeneic PBMC as 'feeder cells', 1% v/v PHA (Gibco) and r-IL-2 (4 U/well) in the above described medium. Growing microcultures were expanded at weekly intervals with fresh feeder cells and r-IL-2. The specificity of TCC was assessed as previously described (Parronchi *et al.*, 1991). Briefly, 2×10^4 T cell blasts were incubated in triplicate cultures in 96-well plates in the presence of 10^5 autologous irradiated (500 rad) PBMC, with whole BP extract and purified n-*Bet v* I. Microtiter plates were then incubated for 48 hr at 37 °C, in 5% CO₂, and in a humidified atmosphere. After pulsing for 16 hr with 0.5 µCi per well of ^3H -thymidine, cells were harvested and the incorporated radioactivity was measured by scintillation counting. When the stimulation index [ratio between counts per min (cpm) obtained in cultures with TCC plus autologous irradiated PBMC plus antigen and cpm obtained in cultures containing TCC and PBMC alone] was greater than 10, responses were considered as positive. Clones with proved specificity for *Bet v* I were retested as described above using BP extract, n-*Bet v* I, r-*Bet v* I, *Cor a* I and *Aln g* I using the same procedure. In all TCC proliferation assays BP extract was used in

a concentration of 20 µg/ml (4 µg/well), the purified allergens were used in 5 µg/well (1 µg/well).

Analysis of the phenotype of TCC

The FITC-labelled monoclonal antibodies (mAb) anti-Leu 4 CD3, anti-Leu 3a CD4, anti-Leu 2a CD8, anti-T cell Antigen Receptor (TCR) $\alpha\beta$ WT31 and anti-TCR $\gamma\delta$ I (Becton-Dickinson, Mountain View, CA) were used to analyse the phenotype of the TCC on a FACScan (Becton-Dickinson).

RESULTS

Purification of recombinant and natural allergens

By the methods described, all four proteins (r-Bet v I, n-Bet v I, Cor a I and Aln g I) could be purified to homogeneity. Protein staining (silver stain) in

SDS-PAGE showed the single bands in the molecular weight range described for these allergens (not shown).

IgE-immunoblots

All seven patients exhibited specific IgE against Bet v I. Natural as well as recombinant Bet v I was used for immunoblotting (Figs 1a, b). In addition, the patients' sera showed comparable IgE-binding to both, purified Cor a I and Aln g I (Figs 1c, d).

Proliferation assays using r-Bet v I, n-Bet v I, Cor a I and Aln g I

Two TCC, cloned from different T cell lines of patient SAZ, and six TCC derived from specific T cell lines of patients AS, BE, DF, HC, MB, SS were investigated. Specificity of TCC for Bet v I was demonstrated in proliferation assays using n-Bet v I as well as r-Bet v I

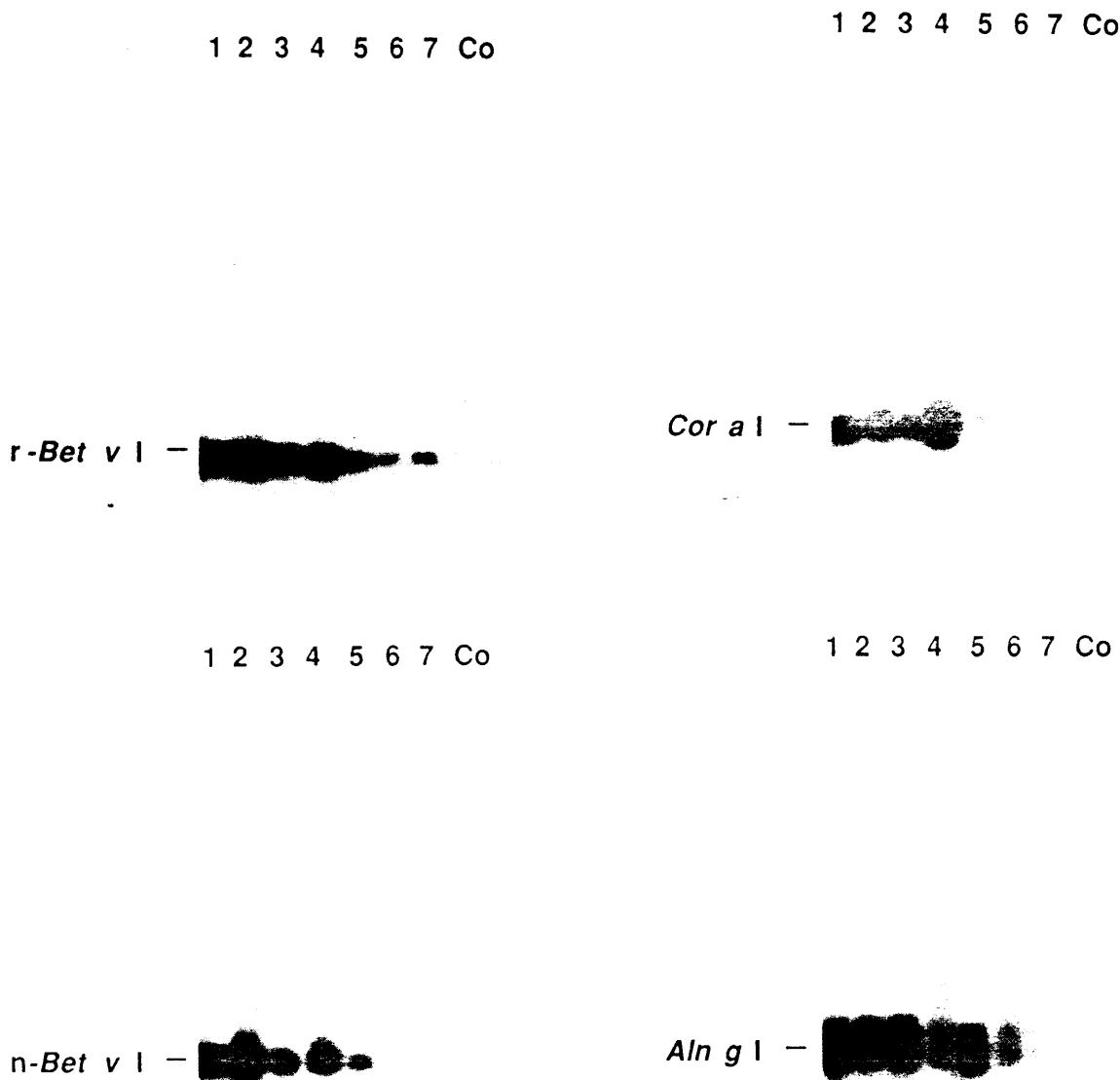


Fig. 1. Immunoblot analysis with purified allergens: the reactivity of patient's IgE with recombinant (r-Bet v I), natural Bet v I (n-Bet v I), Cor a I and Aln g I is compared. (1)–(7) Patients sera: HC, MB, SAZ, SS, AS, DF, BE. Co represents the control, a serum pool of 13 non-allergic donors.

Table 1. Proliferation assays

Clone	³ H-thymidine incorporation (cpm × 10 ³)							
	AS27	BE15	DF16	HC5	MB20	SS10	SAZ4 1	SAZ28 2
Control ^a	0.1	0.06	0.4	0.2	0.2	2.6	0.2	1.2
BP	8	73.6	58.7	106.8	17.5	137.2	12.7	24.9
r-Bet v I	4.5	75.7	3.0	87.7	0.8	80.7	ND	ND
n-Bet v I	5.7	66.8	32.2	90.4	6.8	133.7	8.1	25.5
Cor a I	10.4	65.9	23.1	57.4	14.7	103	11.6	26.8
Aln g I	9.3	28.7	9.2	28.5	10.5	167	7.5	29.3

^aTCC + autologous irradiated PBMC, no antigen.

(Table 1). Stimulation of these eight TCC with *Cor a I* and *Aln g I* in the same concentration also led to strong proliferative responses. The reactivity patterns were quite different: 5/8 *Bet v I*-specific TCC reacted distinctly more strongly upon stimulation with *Cor a I* than with *Aln g I* (BE15, DF16, HC5, MB20, SAZ 4/1; Table 1), 1/8 clone (SS10; Table 1) showed stronger reactivity with *Aln g I*. In two TCC (MB20, AS27; Table 1) the reactivity to the cross-reacting proteins seemed to be even more pronounced than the response to *Bet v I*. One clone (MB20) did not react with the r-*Bet v I* allergen.

The results of the proliferation assays are summarized in Table 1.

Proliferation assays using epitope-representing peptides

Three clones were tested with peptides representing the epitope on *Bet v I* and homologous peptides on *Cor a I* and *Aln g I*, respectively. Each of the TCC reacted strongly with the peptides derived from these sources (Fig. 2). TCC HC5 displayed a 10-fold weaker proliferation following stimulation with the *Aln g I* derived peptide, than with the *Bet v I* and *Cor a I* derived

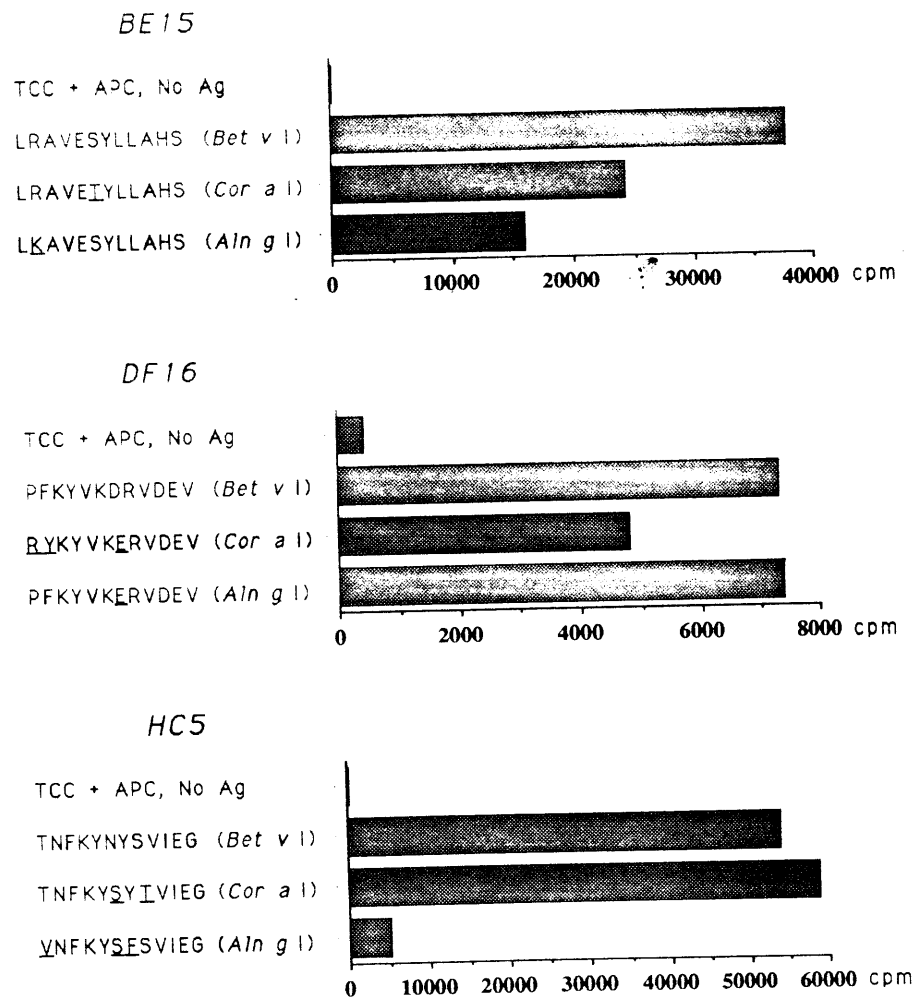


Fig. 2. Response of three representative TCCs to stimulation with peptides synthesized according to the *Bet v I*, *Cor a I* and *Aln g I* sequence. The letters represent amino acids in the international one-letter code.

peptide: *Bet v I*⁷⁷⁻⁸⁸: 53,872 cpm, *Cor a I*⁷⁷⁻⁸⁸: 58,667 cpm, *Aln g I*⁷⁷⁻⁸⁸: 5036 cpm (background value: 216 cpm).

Flow cytometry analysis

FACS analysis revealed the helper cell phenotype (CD3+, CD4+, CD8-) for all clones. The TCR could be identified in all cases as TCR α β .

DISCUSSION

It has been demonstrated that *Bet v I*, the major allergen of BP shares IgE-binding epitopes with tree pollen allergens derived from pollen of other trees of the order *Fagales* (Ipsen *et al.*, 1985; Ipsen and Hansen, 1991; Rohac *et al.*, 1991; Valenta *et al.*, 1991a), and that a molecule homologous to *Bet v I* is present in different fruits and vegetables (Halmepuro *et al.*, 1984; Ebner *et al.*, 1991). *Bet v I* is an important major tree pollen allergen. More than 95% of all tree pollen allergic patients exhibit IgE to this protein and 60% react exclusively with this allergen (Jarolim *et al.*, 1989a). Thus, this protein is very important for diagnosis and therapy of tree pollen allergy (Valenta *et al.*, 1991b). In this respect, the definition of T cell specificities and cross-reactivities seems to be essential. In this study, *Bet v I*-specific TCC were used to investigate T cell epitopes common to the major allergens of birch, hazel and alder pollens, since these major tree pollen allergens are known to cross-react at the B cell epitope level.

In the last few years, new cell culture techniques have provided us with information about the particular response of allergen-specific T helper lymphocytes upon stimulation with allergens. The CD4+ T cells could be divided into several subsets according to their pattern of lymphokine production (Mosmann and Coffman, 1989; Chrétien *et al.*, 1990; Maggi *et al.*, 1992). Also, a pathway of IgE regulation, mainly depending on the ratio of IL-4 and IFN γ production by these T helper cells was described for mice (Mosmann and Coffman, 1989) and could recently also be shown for humans (Chrétien *et al.*, 1990; Maggi *et al.*, 1992). Immunotherapy that means inducing tolerance in an allergic patient by application of increasing doses of allergen, is an approved method in the treatment of type I allergy. The effectiveness of this method is well known (Bousquet *et al.*, 1991), however, the mechanisms taking place are not completely understood. Many studies reported no correlation between therapy success and changes in the levels of antibodies of whichever class or subclass (Birkner *et al.*, 1990). It is conceivable that during immunotherapy a modulation of the immune response of allergen specific T-helper cells takes place. Severe cases of tree pollen allergy are treated by hyposensitization using mixtures of representative tree pollen extracts (normally a mixture of birch, hazel and alder extract) to which patients display specific IgE (Nüchle-Petersen *et al.*, 1988). In our opinion it is important also to investigate such cross-reactivities at the T cell level. To avoid errors we used highly specific systems like *Bet v I* specific T cell clones and purified natural allergens and

additionally, in case of *Bet v I*, a recombinant protein. Our experiments revealed common T cell epitopes on *Bet v I*, *Cor a I* and *Aln g I*. Each of the eight T cell clones tested, reacted with all three major allergens, but with different intensity (Table 1). This was probably due to the fact, that distinct clones recognize different T cell epitopes (Yssel *et al.*, 1992; Ebner *et al.*, 1993a, b) in the amino acid sequence of the protein. To answer this question, peptides were synthesized according to three immunodominant T cell epitopes of *Bet v I*, and TCCs reacting with the respective sequences were tested with the corresponding parts of *Cor a I* and *Aln g I*. Proliferation assays revealed that the amino acid changes taking place in these 12-mer peptides were not essential. However, clone HC5 reacted with the *Aln g I*-derived peptide positively (5036 vs 216 cpm in the negative control), but 10-fold weaker than with the birch or hazel-derived peptide (Fig. 2). In this case, three amino acid changes could be observed within the T cell epitope (Thr vs Val, Asp/Tyr vs Ser/Phe). The peptide synthesized according to the published sequence of *Aln g I* (Breiteneder *et al.*, 1992) also displayed weaker antigenicity than purified natural *Aln g I*, indicating that this allergen preparation comprises a mixture of isoforms with different antigenicity (i.e. amino acid changes within particular T cell epitopes; Breiteneder *et al.*, 1993). It has been described in the literature that distinct amino acids of an epitope are crucial either for peptide-MHC class II or peptide-TCR-binding (Allen *et al.*, 1990; Suhrbier *et al.*, 1991; Krieger *et al.*, 1991). Within the sequences of the three molecules, minor changes in peptides representing T cell epitopes could reduce or increase specific T cell affinity. One of the tested clones (MB20; Table 1) showed reactivity to n-*Bet v I*, to which this TCC was originally raised, and responded even stronger to the hazel and alder proteins. Surprisingly, this clone did not react with r-*Bet v I* in all the experiments performed (Table 1). We believe that a crucial amino acid change within the T cell epitope prevents reactivity with the particular isoform represented by our recombinant material. This stimulating sequence is obviously present in the mixture of isoforms in the natural preparations of *Bet v I*, *Cor a I* and *Aln g I*. Interestingly, the patients' IgE bound to the r-*Bet v I* (lane 2, Fig. 1a).

In conclusion, a complete characterization of allergenic molecules that considers B as well as T cell epitopes is necessary. Moreover, an involvement of particular MHC class II restriction elements (Fischer *et al.*, 1992; Ansari *et al.*, 1989) and TCR molecules (Renz and Gelfand, 1992) in reactivity of T helper cells with immunodominant (cross-reacting) epitopes has to be investigated. This knowledge might provide us with new insights into mechanisms leading to tolerance (IT) and disclose how specific T cells recognize structures on allergenic proteins. Single proteins (e.g. recombinant allergens) should be used in the therapy of type I allergy. In future, peptides representing epitopes of allergens (maybe also present on other cross-reacting proteins showing amino acid sequence homology) could also be good tools for IT (Berzofsky *et al.*, 1987; Litwin *et al.*,

1991). They could modulate the immune response to allergens at the T cell or B cell level without the risk of anaphylactic side effects.

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REFERENCES

- Allen P., Matsueda G., Adams S., Freeman J., Roof R., Lambert L. and Unanue E. (1990) Enhanced immunogenicity of a T cell immunogenic peptide by modifications of its N and C termini. *Int. Immun.* **1**, 141–150.
- Amann E., Brosius J. and Ptashne M. (1983) Vectors bearing a hybrid trp-lac promoter useful for regulated expression of cloned genes in *Escherichia Coli*. *Gene* **25**, 165–178.
- Ansari A. A., Friedhoff L. R., Meyers D. A., Bias W. B. and Marsh D. G. (1989) Human immune responsiveness to *Lolium perenne* pollen allergen *Lol p* III (Rye III) is associated with HLA-DR3 and DR5. *Hum. Immun.* **25**, 59–71.
- Berzofsky J. A., Cease K. B., Cornette J. L., Spouge J. L., Mergalit H., Berkower I. J., Good M. F., Miller L. H. and De Lisi C. (1987) Protein antigenic structures recognized by T cells: Potential applications to vaccine design. *Immun. Rev.* **98**, 9–52.
- Birkner T., Rumpold H., Jarolim E., Ebner H., Breitenbach M., Skvaril F., Scheiner O. and Kraft D. (1990) Evaluation of immunotherapy-induced changes in specific IgE, IgG and IgG subclasses in birch pollen allergic patients by means of immunoblotting. Correlation with clinical response. *Allergy* **45**, 418–426.
- Bousquet J., Becker W. M., Hejjaoudi A., Chanal I., Lebel B., Dhivert H. and Michel F. B. (1991) Differences in clinical and immunologic reactivity of patients allergic to grass pollen and to multiple-pollen species. II. Efficacy of a double blind, placebo controlled, specific immunotherapy with standardized extracts. *J. Allergy Clin. Immun.* **88**, 43–53.
- Breiteneder H., Pettenburger K., Bito A., Kraft D., Rumpold H., Scheiner O. and Breitenbach M. (1989) The gene coding for the major birch allergen, *Bet v* I, is highly homologous to a pea disease resistance response gene. *EMBO J.* **8**, 1935–1938.
- Breiteneder H., Ferreira F., Hoffmann-Sommergruber K., Ebner C., Breitenbach M., Rumpold H., Kraft D. and Scheiner O. (1993) Four recombinant isoforms of *Cor a* I, the major allergen of hazel pollen, show different IgE-binding properties. *Eur. J. Biochem.* **212**, 355–362.
- Breiteneder H., Ferreira F., Reikersdorfer A., Duchene M., Valenta R., Hoffmann-Sommergruber K., Ebner C., Breitenbach M., Rumpold H., Kraft D. and Scheiner O. (1992) cDNA cloning and expression in *Escherichia coli* of *Aln g* I, the major allergen of alder pollen (*Alnus glutinosa*). *J. Allergy Clin. Immun.* **90**, 909–917.
- Chrétien I., Pène J., Brière F., De Waal Maljeff R., Rousset F. and De Vries J. E. (1990) Regulation of IgE synthesis. I. Human IgE synthesis *in vitro* is determined by the reciprocal antagonistic effects of interleukin 4 and interferon- γ . *Eur. J. Immun.* **20**, 343–351.
- Ebner C., Szépfalusi Z., Ferreira F., Valenta R., Parronchi P., Maggi E., Romagnani S., Scheiner O. and Kraft D. (1993a) Identification of multiple T cell epitopes on *Bet v* I, the major birch pollen allergen, using allergen-specific T cell clones and overlapping peptides. *J. Immun.* **150**, 1047–1054.
- Ebner C., Schenk S., Szépfalusi Z., Hoffmann K., Ferreira F., Willheim M., Scheiner O. and Kraft D. (1993b). Multiple T cell specificities for *Bet v* I, the major birch pollen allergen, within single individuals. Studies using specific T cell clones and overlapping peptides. *Eur. J. Immun.* **23**, 1523–1527.
- Ebner C., Birkner T., Valenta R., Rumpold H., Breitenbach M., Scheiner O. and Kraft D. (1991) Common epitopes of birch pollen and apples—studies by Western and Northern blot. *J. Allergy Clin. Immun.* **88**, 588–594.
- Ferreira F., Pettenburger K., Hoffmann-Sommergruber K., Breiteneder H., Ebner C., Szépfalusi Z., Steiner R., Bohle B., Rumpold H., Kraft D. and Scheiner O. (1992) Purification of recombinant and birch pollen derived *Bet v* I: comparison of immunological properties. In *Immunology of Allergens* (Edited by D. Kraft and A. Sehon), pp. 205–208. CRC Press.
- Fischer G. F., Pickl W. F., Fae I., Ebner C., Ferreira F., Breiteneder H., Vlkoukal E., Scheiner O. and Kraft D. (1992) Association between IgE response against *Bet v* I, the major allergen of birch pollen, and HLA-DRB alleles. *Hum. Immun.* **33**, 259–265.
- Halmepuro L., Vuontela K., Kalimo K. and Björkstén F. (1984) Crossreactivity of IgE antibodies with allergens in birch pollen, fruits and vegetables. *Int. Arch. Allergy Appl. Immun.* **74**, 235–240.
- Ipsen H., Bowadt H., Janniche H., Nuechel Petersen B., Munch E. P., Wihl J. A. and Loewenstein H. (1985) Immunochemical characterization of reference alder (*Alnus glutinosa*) and hazel (*Corylus avellana*) pollen extracts and the partial immunochemical identity between the major allergens of alder, birch, and hazel pollen. *Allergy* **40**, 510–518.
- Ipsen H. and Hansen O. C. (1991) The NH₂-terminal amino acid sequence of the immunochemically partial identical major allergens of alder (*Alnus glutinosa*) *Aln g* I, birch (*Betula verrucosa*) *Bet v* I, hornbeam (*Carpinus betulus*) *Car b* I and oak (*Quercus alba*) *Que a* I pollen. *Mol. Immun.* **11**, 1279–1288.
- Jacobs S. (1959) Determination of nitrogen in proteins by means of indanetrione hydrate. *Nature* **183**, 262.
- Jarolim E., Rumpold H., Endler A. T., Ebner H., Scheiner O. and Kraft D. (1990) IgE and IgG antibodies of patients with allergy to birch pollen as tools to define the allergen profile of *Betula verrucosa*. *Allergy* **44**, 385–395.
- Jarolim E., Tejki M., Rohac M., Schlerka G., Scheiner O., Kraft D., Breitenbach M. and Rumpold H. (1989b) Monoclonal antibodies against birch pollen allergens: characterization by immunoblotting and use for single-step affinity purification of the major allergen *Bet v* I. *Int. Arch. Appl. Immun.* **90**, 54–60.
- Krieger J. I., Karr R. W., Grey H. M., Yu W. Y., O'Sullivan D., Batovsky L., Zheng Z. L., Colón S. M., Gaeta F. C. A., Sidney J., Albertson M., Del Guercio M. F. and Chestnut R. W. and Sette A. (1991) Single amino acid changes in DR and antigen define residues critical for peptide-MHC binding and T cell recognition. *J. Immun.* **146**, 2331–2340.
- Litwin A., Pesce A. J., Fischer T., Michael M. and Michael J. G. (1991) Regulation of the human response to ragweed pollen by immunotherapy. A controlled trial comparing the effect of immunosuppressive peptic fragments of short ragweed with standard treatment. *Clin. Exp. Allergy* **21**, 457–465.
- Maeji N. J., Bray A. M. and Geysen H. M. (1990) Multi-pin peptide synthesis strategy for T cell determinant analysis. *J. Imm. Methods* **134**, 23–29.

- Maggi E., Parronchi P., Manetti R., Simonelli C., Piccini M. P., Rugiu F. S., de Carli M., Ricci M. and Romagnani S. (1992) Reciprocal regulatory effects of IFN γ and IL-4 on the *in vitro* development of human Th1 and Th2 clones. *J. Immun.* **148**, 2142-2147.
- Merril C., Goldman D., Sedman S. A. and Ebert M. H. (1981) Ultrasensitive staining for proteins in polyacrylamide gels shows regional variation in cerebrospinal fluid proteins. *Science* **211**, 1437-1438.
- Mosmann T. R. and Coffman R. L. (1989) Heterogeneity of cytokine secretion patterns and function of helper T cells. *Adv. Immun.* **46**, 111-147.
- Nüchel-Petersen B., Janniche H., Munch E. P., Wihl J. A., Bowadt H., Ipsen H. and Loewenstein H. (1988) Immunotherapy with partially purified and standardized tree pollen extracts. I. Clinical results from a three-year double blind study of patients treated with pollen extracts either of birch or combinations of alder, birch and hazel. *Allergy* **43**, 353-362.
- Parronchi P., Macchia D., Picchini M. P., Biswas P., Simonelli C., Maggi E., Ricci M., Ansari A. A. and Romagnani S. (1991) Allergen and bacterial antigen-specific T cell clones established from atopic donors show a different profile of cytokine production. *Proc. natn. Acad. Sci. U.S.A.* **88**, 4538-4542.
- Renz H. and Gelfand E. W. (1992) T-cell receptor V elements regulate murine IgE production and airway responsiveness. *Allergy* **47**, 270-276.
- Rohac M., Birkner T., Reimitzer I., Bohle B., Steiner R., Briesenbach M., Kraft D., Scheiner O., Gabl F. and Rumpold H. (1991) The immunological relationship of epitopes on the major tree pollen allergens. *Mol. Immun.* **28**, 897-906.
- Suhrbier A., Rodda S. J., Ho H. C., Csurses P., Dunckley H., Saul A., Geysen M. H. and Rzepezyk C. M. (1991) Role of single amino acids in the recognition of a T cell epitope. *J. Immun.* **147**, 2507-2513.
- Valenta R., Breiteneder H., Pettenburger K., Breitenbach M., Rumpold H., Kraft D. and Scheiner O. (1991a) Homology of the major birch-pollen allergen, Bet v I, with the major pollen allergens of alder, hazel and hornbeam at the nucleic acid level as determined by cross-hybridization. *J. Allergy Clin. Immun.* **187**, 677-682.
- Valenta R., Duchene M., Vrtala S., Birkner T., Ebner C., Hirschwehr R., Breitenbach M., Rumpold H., Scheiner O. and Kraft D. (1991b) Recombinant allergens for immunoblot diagnosis of tree pollen allergy. *J. Allergy Clin. Immun.* **88**, 889-894.
- Yssel H., Johnson K. E., Schneider P. V., Wideman J., Terr A., Kastelein R. and de Vries J. E. (1992) T cell activation-inducing epitopes of the house dust mite allergen Der p I. *J. Immun.* **148**, 738-745.