



SEQUENCE COMPARISONS OF THE CDR3 HYPER-VARIABLE LOOPS OF HUMAN T CELL RECEPTORS SPECIFIC FOR THREE MAJOR T CELL EPITOPES OF THE BIRCH POLLEN ALLERGEN Bet v 1

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Abstract—We have analysed the T cell receptor (TCR) α and β chain sequences of 16 human CD4⁺ T cell clones (TCCs) specific for three important epitopes of the major birch pollen allergen Bet v 1. The TCCs were raised from the peripheral blood of eight patients with birch pollen allergy, showing allergic rhino-conjunctivitis and allergic asthma. The TCCs from these individuals were specific for Bet v 1-derived peptides: amino acids (aa)77–92 (epitope 1), aa93–108 (epitope 2) and aa113–126 (epitope 3). The DNA sequence analysis of the TCRAV and BV regions revealed heterogeneous repertoires for recognition of the peptides. Multiple combinations of AV/AJ and BV/BJ were used. However, some inter-individual restriction was evident. A limited selection of AV8 and the normally infrequently used BV1S4 was obvious in TCCs specific for epitope 1. The TCRBV13 was more frequent in TCCs recognizing epitope 3. A very narrow distribution in length could be seen in the CDR3 sequences of the β chain of TCRs with specificity for epitopes 1 and 2. Inter-individual positional micro-restriction was observed for the aa motif LR in the β CDR3 (epitope 1), for the aa residue M in the α CDR3 and for the aa residue G in the β CDR3 (epitope 3). Our results illustrate clearly that each antigenic peptide derived from a single allergen, is capable of selecting different characteristics in the responding repertoire of TCRs, thus increasing the complexity of allergen-recognition by T lymphocytes. Therefore, our findings limit the potential use of TCR targeted therapeutical strategies in Type I allergy. Copyright © 1996 Elsevier Science Ltd.

Key words: T cell receptor $\alpha\beta$, allergenic peptides, Bet v 1, CDR3, tree pollen allergy.

INTRODUCTION

The IgE-mediated Type I allergy to tree pollen is very common in developed countries of the northern hemisphere. The production of IgE specific for certain pollen proteins by allergic individuals represents an aberrant immune response. The CD4⁺ T lymphocytes (Th2-like) play a substantial role in the regulation of this IgE production (Coffman and Carty, 1986; Chrétien *et al.*, 1990). Molecular cloning techniques and the availability of amino acid sequences of allergenic proteins made the identification of T cell epitopes possible (Chua *et al.*,

1988; Breiteneder *et al.*, 1989; Perez *et al.*, 1990; Larsen *et al.*, 1992; Ebner *et al.*, 1993a; Yssel *et al.*, 1992; Van Neerven *et al.*, 1993). It could be demonstrated that multiple T cell epitopes were distributed over the complete sequence of an atopic allergen (Yssel *et al.*, 1992; Van Neerven *et al.*, 1993; Dhillon *et al.*, 1992; Carballido *et al.*, 1993; Ebner *et al.*, 1993b,c; Van Neerven *et al.*, 1994; Spiegelberg *et al.*, 1994; Higgins *et al.*, 1994; Schenk *et al.*, 1995). Some of these were recognized by T cells from almost every individual sensitized to the particular allergen from which the epitope peptide was derived. Therefore, these epitopes were designated “major epitopes” (Ebner *et al.*, 1993b, c; Schenk *et al.*, 1995).

More than 90% of mature peripheral blood T lymphocytes utilize TCR molecules composed of α and β polypeptide chains, for specific antigen recognition (Kronenberg *et al.*, 1986; Matis, 1990). Although the three-dimensional structure of the TCR has not been determined yet, it has been proposed that it will closely resemble that of immunoglobulins, containing three or four hyper-variable loops that correspond to the complementarity determining regions (CDRs) (Clothia *et al.*,

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Abbreviations: TCL, T cell line; TCC, T cell clone; TCR, T cell receptor; CDR, complementarity-determining region; APC, antigen presenting cell; aa, amino acid(s).

1988). Based on the homology of these hypervariable loops with the Ig CDRs and on molecular modeling, it has been proposed that the CDR1 and -2 loops (encoded by the germline TCRV sequences) interact with the α -helices of the MHC molecules (Clothia *et al.*, 1988; Claverie *et al.*, 1989; Prochnicka-Chalufour *et al.*, 1991). The CDR3 loops, where the highest variability occurs, are encoded by the V-(D)-J junctional regions and are believed to be involved in the interaction with the peptide bound in the groove of the MHC on the APC (Clothia *et al.*, 1988; Davis and Bjorkman, 1988). As the MHC molecule selects the structurally less diverse CDR1 and -2 regions of the TCR, the peptide can be considered the single most important structure responsible for the specificity of the TCR. Sequence information about CDR3 regions of TCRs where the epitope is known, will help to explain the interactions between T cells and APCs.

Several studies in the human system describe TCR selection in the context of known epitope peptides and MHC molecules. Giegerich *et al.* (1992) studied the diversity of α and β chains of TCRs involved in the human autoreactivity to myelin basic protein (MBP), and found that even TCCs recognizing the same MBP peptide bound to identical or very similar HLA-DR molecules, expressed diverse TCRs. By contrast, the HLA-A2.1 restricted response to the influenza A virus matrix peptide 57–68 by cytotoxic T lymphocytes showed that the usage of AV and BV sequences was remarkably conserved, as were certain junctional sequences of both chains (Moss *et al.*, 1991).

The T cell recognition of a tetanus toxin-derived peptide in donors of different HLA-DR haplotypes was characterized by a preferential use of a particular TCRBV gene segment but a heterogeneous TCRAV usage. There was also an apparent lack of CDR3 conservation which was interpreted as adaptation to different MHC molecules (Boitel *et al.*, 1992). In their study, Henwood *et al.* (1993) characterized the TCRs used by TCCs isolated from joints of arthritis patients (Henwood *et al.*, 1993). The clones were restricted by either HLA-DR3 or DP4 and recognized two different epitopes within the M_r 65 000 heat-shock protein (hsp65) of *Mycobacterium leprae*. Although restriction in the use TCRAV and BV segments was found, conservation of the α or β chain CDR3 sequence was not apparent. Jones *et al.* (1994) examined the TCR usage of human CD4⁺ TCCs specific for a T cell epitope of influenza virus hemagglutinin in the context of HLA-DR and -DQ. Only the junctional regions encoding the CDR3 regions were different.

Hawes *et al.* (1995) analysed the TCR- $\alpha\beta$ repertoire specific for a given peptide/MHC complex by using pairs of HLA-identical individuals. They observed that, although the overall TCR- $\alpha\beta$ repertoires were diverse, an intra-individual limited restriction was evident. Moreover, a limited conservation of the use of certain V region genes in the response to the different peptides was found. Wedderburn *et al.* (1993) published the first study of TCR usage of allergen-specific TCC isolated from atopic individuals. Although unfractionated extracts of house dust mites were used, oligoclonality of the TCCs studied

was revealed. However, there was minimal conservation among CDR3 sequences.

Data on TCRs from epitope-mapped T cells specific for atopic allergens has been available only for a minimal epitope (aa144–153) of Bet v 1, the major birch pollen allergen (Breiteneder *et al.*, 1995). Despite the heterogeneity in length and aa composition, all β CDR3s showed an arginine residue at the N-terminal end of the loops.

In this study, we characterized the TCRs of a panel of TCCs isolated from individuals allergic to Bet v 1, the major allergen from birch (*Betula verrucosa*) pollen (Breiteneder *et al.*, 1989). These clones were restricted by HLA-DR and each recognized one of three major epitopes within the M_r 17 000 Bet v 1 allergen. We wanted to evaluate the possible use of TCR sequences as targets for immunotherapy.

MATERIALS AND METHODS

Donor selection

Blood was obtained from eight birch pollen-allergic, unrelated adult donors (one female, seven males). All atopic individuals showed typical case histories, positive skin prick tests and their sera displayed IgE-binding to Bet v 1, the major birch pollen allergen.

Bet v 1 and synthetic Bet v 1-peptides

Recombinant Bet v 1 was expressed in *Escherichia coli* BL21(DE3) cells as a non-fusion protein and purified as described (Ferreira *et al.*, 1993). A panel of 75 overlapping peptides, synthesized by Cambridge Research Biochemicals Ltd (Cambridge, U.K.) according to the Bet v 1 sequence, was used for epitope mapping of TCCs as described previously (Ebner *et al.*, 1993b). In order to make direct comparisons possible, peptide concentrations of 0.75 μ M were applied in all experiments.

Allergen-specific T cell lines and epitope-specific T cell clones

Short term allergen-specific T cell lines (TCL) were generated from PBMC of birch pollen-allergic donors by stimulation with Bet v 1 (10 μ g/ml; 24). The T cell blasts from Bet v 1-specific TCL were seeded in limiting dilution (0.3 cells/well) into 96-well round bottom plates (Nunc, Nunc, Denmark) in the presence of 10⁵ irradiated (5000 rad) allogeneic PBMC as "feeder cells", 1% PHA v/v (Gibco, Grand Island, NY, U.S.A.) and rIL-2 (4 U/well) in the medium. Growing microcultures were then expanded weekly by turns of specific (Bet v 1) and non-specific (rIL-2) stimulation. The specificity of the TCCs was assessed as previously described (Ebner *et al.*, 1993b). Briefly, 2–5 $\times$ 10⁴ T cell blasts were incubated in triplicate cultures in 96-well plates, in the presence of 10⁵ autologous irradiated PBMC with (i) total birch pollen extract; (ii) purified nBet v 1; (iii) rBet v 1; and (iv) overlapping Bet v 1-peptides. Microtiter plates were then incubated for 48 hr at 37 °C, 5% CO₂, in a humidified atmosphere. The specificity of T cell lines and clones was

determined by a standard [^3H]thymidine incorporation assay. When the stimulation index exceeded 10, responses were considered positive. Epitope mapping was performed as described (Ebner *et al.*, 1993b) and TCCs specific for three major epitopes were selected for the study of their TCRs.

Flow cytometry analysis of TCC phenotype

The phenotype of the TCCs was examined by flow cytometry using a FACScan (Becton–Dickinson, Mountain View, CA, U.S.A.) as previously described (Ebner *et al.*, 1993b).

HLA typing and restriction

The HLA typing of patients was performed as described previously (Fischer *et al.*, 1992). In order to identify the HLA molecules involved in antigen presentation, murine monoclonal antibodies against human MHC class II determinants L 243 (anti-HLA-DR (IgG2a)), Leu 10 (anti-HLA-DQ (IgG1)) and B7/21 (anti-HLA-DP (IgG1)) were purchased from Becton–Dickinson. Proliferation assays in the presence of blocking antibodies were performed in 96-well plates as described previously (O'Hehir *et al.*, 1988).

First strand cDNA synthesis and PCR amplification

The total RNA of TCCs was isolated from 1×10^6 cells by the single step guanidinium–isothiocyanate–phenol–chloroform extraction method (Chomczynski and Sacchi, 1987). Single-stranded cDNA synthesis was carried out on 1.2–1.8 μg of total RNA, with oligo(dT)_{12–18} and avian myeloblastosis virus reverse transcriptase. TCR α and β cDNAs were directly subjected to DNA amplification by PCR using Amplitaq[®] DNA Polymerase (Perkin Elmer, Norwalk, CT, U.S.A.) with oligonucleotide primers complementary to TCR V and C region sequences (Panzara *et al.*, 1992; Genevée *et al.*, 1992), at a final concentration of 0.25 μM . Reactions were incubated in a DNA thermal cycler (Perkin Elmer). Reaction mixtures were subjected to the following conditions: denaturation at 93°C for 1 min, annealing at 55°C for 30 sec and extension

at 72°C for 1 min, with a further 5 sec extension after each cycle.

Cloning and sequence analysis of TCR α and β chains

The PCR reaction products were cloned into the TA cloning vector pCRII[™] (Invitrogen Corporation, San Diego, CA, U.S.A.) and sequenced by the enzymatic chain termination method (Sanger *et al.*, 1977), using a Sequenase Version 2.0 kit (United States Biochemical Corporation, Cleveland, OH, U.S.A.). For each of the TCR α and β chains studied, three independent pCRII clones were sequenced to identify possible sequence errors caused by nucleotide mis-incorporation by the Taq polymerase. Sequences were compared with gene data bank entries (Genbank, Los Alamos, NM, U.S.A.; EMBL, Heidelberg, Germany) and assigned to available published TCR V and J segments (Koop *et al.*, 1994; Moss *et al.*, 1993; Roman-Roman *et al.*, 1991; Wilson *et al.*, 1988; Kimura *et al.*, 1986, 1987; Klein *et al.*, 1987; Yoshikai *et al.*, 1986; Ferradini *et al.*, 1991; Toyonaga *et al.*, 1985). Virtually all TCRV transcripts have the amino acid sequence Y-(L/F)-C-A (α chain) or Y-(L/F)-C-A with two additional serine residues in most cases (β chain) at the 3' end of the V gene segment and the motif F-G-X-G-T within the J segment (Moss and Bell, 1995). The CDR3 length was taken as the number of amino acids between the last germline encoded residue of the V segment and the conserved phenylalanine residue.

RESULTS

T cell clones and epitope mapping

Sixteen TCCs derived from eight birch pollen-allergic individuals were included in this study. All clones revealed the helper cell phenotype (CD3⁺, CD4⁺) and expressed the $\alpha\beta$ TCR. The HLA typing of the donors revealed the MHC class II types, shown in Table 1. Using the antibody L243, which recognizes a determinant common to all DR molecules, proliferation in response to stimulation with Bet v I could be blocked in all TCCs in

Table 1. The HLA-DR and -DQ haplotypes of the donors and numbers of independent TCCs per Bet v I derived epitope–peptide

Donor HLA MHC class II	TCCs/epitope no.		
	1	2	3
XPAA DRB1* 13, 13; DRB 3	—	—	1
XPAS DRB1* 07, 14; DRB 3; DRB 4	—	—	2
XPBP DRB1* 11, 16; DRB 3; DRB 5	2	—	—
XPGZ DRB1* 07, 08; DRB 4	1	—	—
XPHC DRB1* 07, 11; DRB 3; DRB 4	2	1	2
XPMS DRB1* 04, 13; DRB 3; DRB 4	—	1	—
XPSZ DRB1* 07, 15; DRB 4	2	1	—
XPTF DRB1* 07, 15; DRB 3; DRB 5	—	1	—
No. of TCCs/epitope	7	4	5

a dose-dependent manner, indicating restriction by HLA-DR molecules.

Each TCC recognized one of three major T cell epitopes of Bet v 1 (Ebner *et al.*, 1993b, c). The first group was composed of seven TCCs from five patients recognizing the Bet v 1 peptide aa77–92, termed epitope 1, the second group contained four TCCs from four patients specific for Bet v 1 epitope 2 (aa 93–108), and the third group consisted of five TCCs from three patients recognizing Bet v 1 epitope 3 (aa113–126). The distribution of the TCCs into the three groups is listed in Table 1.

TCR α and β chain analysis of epitope-specific TCCs

Epitope 1 (for an overview see Table 2)

TCRA (Fig. 1(A)). The TCCs 53 I and 173 I from the patient XPBP used the closely related TCRAV subfamilies 2S1 and 2S2, but differed in their TCRAJ segments. Three of the seven TCCs used members of the TCRAV8 family, two TCCs used TCRAV 12S1 and one TCC used TCRAV17S1. The 12S1 segments were combined with fairly long CDR3 regions in both TCCs studied (XPHC 5 I and XPSZ 31 II), i.e. 15 or 14 amino acid residues, respectively. The 8S1 segments were joined to shorter CDR3 regions ranging from 10 to 12 amino acid residues (Fig. 1(A)). The shortest CDR3 regions were found combined with the TCRAV2 segments (XPBP 53 I and 173 I). In general, the length of the CDR3 loop of the TCR α chains had a fairly wide distribution with

sequences between eight and 15 amino acids. The clone XPSZ 31 II had two different mRNA species coding for functionally rearranged TCR α chains.

TCRB (Fig. 1(B)). The TCCs 53 I and 173 I from patient XPBP used the same 2S3 segments in the V regions and the same J segments 1S4 in their CDR3 regions. At the N-terminal end of the CDR3 sequence, both clones showed an aspartic acid residue. The TCCs 5 I and 8 III from patient XPHC used the closely related subfamilies 5S1 and 5S2/3, but differed in their J segments. Although the use of V segments among the TCCs studied was diverse, there was a bias towards J1S4 (4/7 TCCs) and J2S7 (2/7 TCCs). The length of the CDR3 loop had a narrow distribution (between nine and 10 aa). A motif consisting of the aa leucine and arginine could be observed in the N-terminal portion of the CDR3s of four TCCs.

Epitope 2 (for an overview see Table 2)

TCRA (Fig. 2(A)). Each TCC used a different AV and AJ segment. No particular selection of amino acid residues could be detected in the CDR3s whose lengths varied between nine and 12 amino acid residues.

TCRB (Fig. 2(B)). Each TCC used V and J segments that differed from the ones detected in the other clones. Two functional β chain mRNAs were detected for XPSZ 97 III, indicating the expression of two discrete and very similar TCRs that only differed slightly in their CDR3s. The usage of the closely related BV segments 5S1 and

Table 2. The V and J segments used by the TCR α and β chains specific for Bet v 1 epitopes 1–3

TCC	TCRAV	Amino acids in CDR3	TCRAJ	TCRBV	Amino acids in CDR3	TCRBD	TCRBJ	TCRBC
Bet v 1 epitope 1 ⁷⁷ TNFKYNYSVIEGGPIG ⁹²								
XPBP 53I	2S2	9	1S4*	2S3	10	1	1S4	1
173I	2S1	8	8	2S3	9	11	1S4	1
XPGZ 17I	8S1	10	45	9S1	10	2	2S3	2
XPHC 5I	12S1	15	58	5S1	9	1	1S4	1
8III	8S1	12	3	5S2/3	10	2	2S7	2
XPSZ 11II	17S1	15	32	6S2/3	10	2	2S7	2
31II	8S2	10	9S7*	12S2	9	1	1S4	1
31II	12S1	14	52					
Bet v 1 epitope 2 ⁹³ DTLEKISNEIKIVATP ¹⁰⁸								
XPHC 2III	5S1	9	21	6S2/3	9	1	1S1	1
XPMS 2I	2S1	12	57	12S2	10	1	1S2	1
XPSZ 97III	18S1	11	15	5S2/3	10	2	2S1	2
				5S2/3	10	2	2S1	2
XPTF 1I	8S1	12	44	5S1	11	2	2S3	2
Bet v 1 epitope 3 ¹¹³ ILKISNKYHTKGDH ¹²⁶								
XPAA 4I	13S1	10	41	14S1	7	2	1S4	2
XPAS 16I	8S1	13	52	6S2/3	10	2	2S7	2
27I	8S2	10	11	13S5	8	1	1S1	1
XPHC 33III	6S1	14	28	13S2	9	2	2S7	2
46IV	2S1	9	34	13S3	9	1	1S2	1
46IV	11S1	8	31					

The TCRAJ segments were assigned according to Koop *et al.* (1994) and Moss *et al.* (1993) (marked with an asterisk).

The TCRAV, BV, BD, and BJ segments were assigned according to sequences previously published using the standardized TCR nomenclature. The α and β CDR3s were defined according to Clothia *et al.* (1988) and their lengths are listed as the number of amino acid residues.

(A)

77TNFKYNYSVIEGGPIG ⁹²					
T cell clone	TCR gene segments	TCRAV	CDR3	TCRAJ	TCRAC
XPBP 53 I	V2S2J1S4* F S D S A T Y L C A M	CCCAGTATTGAGCCACCTCTGTGCAATG F S D S A T Y L C A M	GGGAAAGCTGGAGGCTTCAAAATATC G E T G G F E C T	TTTGGAGCAGGAACAGACTATTGTTTAAAGCA F G A G T R L L F V K A	AATATCCAG N I Q
173 I	V2S1J8C S D S A T Y L C A V	AGTGATCAGCCACTTACCTGTGCGGTG S D S A T Y L C A V	AACACAGGCTTACAGAACTTTGA N T G F E Q S L V	TTTGGAACTGGACCCGACTTCTGTCAGTCCA F G T G T R L L V S P	AATATCCAG N I Q
XPBZ 17 I	V8S1J45C E D S A V Y F C A	GAGACTCGGCTGTCTACTCTGTGCA E D S A V Y F C A	GTTAGCGGAGGAGTGTGACCGACTCAGC V R G G G A D R L L C	TTTGGCAAGGGAAGTCACTAATCATCCAGCC F G K G T H L I I Q P	TATATCCAG Y I Q
XPHC 5 I	V12S1J56C V D S A V Y F C A L	GTGGACTCAGCAGTACTCTGTGCTCG V D S A V Y F C A L	GAGTCCTACTACTGATTAAAGGAACAGTGGCTTAGCTTACG E S L L L I K E T S G S R L T	TTTGGGGAAGGAACAGCTCAGACGTAATCT F G E G T Q L T V N P	GATATCCAG E I Q
8 III	V8S1J3C E D S A V Y F C A	GAGACTCGGCTGTCTACTCTGTGCA E D S A V Y F C A	GCAAAGCGGGTACAGCAGTCTTCCAAAGATATC A K T G Y S S A S V I I	TTTGGATCAGGGAACAGACTCAGATCCGACCA F G S G T R L S I R P	AATATCCAG N I Q
XPSZ 11 II	V17S1J32C G D S A V Y F C A A	GGAGACTCTGAGTGTACTCTGTGCAACA G D S A V Y F C A A	GAGAGTCTGTAGCACTAAGTGCTGTCTACAAACAGCTATC D E F V R R Y G G A T N K L I	TTTGGAACTGGCACTCTGCTGCTCCAGCCA F G T G T L L A V V P	AATATCCAG Y I Q
31 II	V8S2J9S7* G D S A V Y F C A E	GGAGACTCAGCTCTCTACTTTTGTGACAG G D S A V Y F C A E	AATACAGAGGAGGCTGTTTACGAGCTCAGC N R G G V Y E I T	TTTGGTAAAGGAGTCACTAATCATCCAGTCC F G K G T H L I I Q S	TATATCCAG Y I Q
31 II	V12S1J52C V D S A V Y F C A	GTGGACTCAGCAGTACTCTGTGCTT V D S A V Y F C A	CCCCACCTCTGCTGCTGCTACTAGTACGAAAGCTGACA P P P R A G G T S Y G R L T	TTTGGCAAGGGAACACTCTGACTGCTCCATCCA F G Q G T I L T V H P	ACATCCAG N I Q

(B)

77TNFKYNYSVIEGGPIG ⁹²					
T cell clone	TCR gene segments	TCRBV	CDR3 (TCRBD)	TCRBJ	TCRBC
XPBP 53 I	V2S3D1J1S4C1 S F Y I C S A R	AGCTTCTACATCTGCGTGTAGA S F Y I C S A R	GATCTCAGGGAAGTAAATGAAAAGCTGTT D L R E T N E K L F	TTTGGCAGTGGAAACCCAGCTCTCTGCTTG F G S G T Q L S V L	GAGGACCTG E D L
173 I	V2S3D1J1S4C1 S F Y I C S A R	AGCTTCTACATCTGCGTGTAGA S F Y I C S A R	GAGCGGCTCACTAATGAAAAGCTGTT D G P T N E K L F	TTTGGCAGTGGAAACCCAGCTCTCTGCTTG F G S G T Q L S V L	GAGGACCTG E D L
XPBZ 17 I	V9S1D2J2S3C2 S A V Y F C A S	TCTGCTGTGTAATCTGTGTCAGC S A V Y F C A S	AGATTGCGCCGTAGCAGATAGCAGTAT R L R R S E T D T G Y	TTTGGCCAGGCAACCCGCTGACAGTCTC F G P G T R L T L L	GAGGACCTG E D L
XPHC 5 I	V5S1D1J1S4C1 A L Y L C A S S	GCCCTTATCTTTGCGCCAGCAGC A L Y L C A S S	ATGGCGGGGCTTTGAAAAGCTGTT M G G A L E K L F	TTTGGCAGTGGAAACCCAGCTCTCTGCTTG F G S G T Q L S V L	GAGGACCTG E D L
8 III	V5S2/3D2J2S7C2 A L Y L C A S S	GCCCTGTATCTCTGTGCGCAGC A L Y L C A S S	TTGAGCAACCCGGGATACGAGCAGTAC L A R N P G Y E Q Y	TTTGGCGGGGCAACCCAGCTCAGGTCACA F G P G T R L T V T	GAGGACCTG E D L
XPSZ 11 II	V6S2/3D2J2S7C2 A V Y L C A S S	GCCCTGTATCTCTGTGCGCAGC A V Y L C A S S	TTAGCTAGAGGAGCTCTCTAGCAGCAGTAC L A R G T L Y E D Y	TTTGGCGGGGATACCCAGCTCAGGTCACA F G P G T R L T V T	GAGGACCTG E D L
31 II	V12S2D1J1S4C1 S V Y F C A I S	TCTGTGTACTTCTGTGCGCAGT S V Y F C A I S	GCAGAGACCTCTAATGAAAAGCTGTT A E T L N E K L F	TTTGGCAGTGGAAACCCAGCTCTCTGCTTG F G S G T Q L S V L	GAGGACCTG E D L

Fig. 1. Nucleotide and deduced amino acid sequences of TCR α (A) and TCR β (B) junctional regions of independent TCCs specific for Bet v 1 epitope 1 (aa77–92). Gene segments were assigned as described in Table 2. For each sequence, only the last 7–10 V gene amino acid residues are shown, followed by the presumed Ig-like loops (CDR3) and by the first three residues of the C region. The underlined CDR3 aa are encoded by the J segments. Nucleotides that represent possible contributions from the TCRBD gene segments are printed in bold type. These sequence data are available from EMBL/GenBank/DBJ under the accession numbers X92766–X92780.

5S2/3 were detected in two of the four TCCs, i.e. XPSZ 97 III and XPTF 1 I. The CDR3 lengths were between nine and 11 amino acids.

Epitope 3 (for an overview see Table 2)

TCRA (Fig. 3(A)). The two clones 16 I and 27 I of patient XPAS which were derived from one cloning procedure used the same AV segment 8S2, but differed in their AJ usage and length of the CDR3s. Each other TCC used different AV and AJ segments, with the CDR3 sequences spanning from eight to 14 amino acid residues. The amino acid methionine appeared in the N-terminal portion of the CDR3s of three out of five clones tested. Two functionally rearranged mRNAs for α chains were detected for clone XPHC 46 IV.

TCRB (Fig. 3(B)). Three of five TCCs tested used closely related BV13 subfamily segments (13S2, 13S3,

13S5). The CDR3 sequences, which ranged between seven and 10 amino acids, were different in amino acid composition for each β chain tested. The TCC XPAA 4 I showed the assembly of a TCRBC2 positive transcript with a J gene from the BJ1 cluster.

Comparison of the CDR3 (N–D–N) regions of TCRs with the same epitope specificity

A limited selection within the amino acid composition of the CDR3 (N–D–N) regions was observed for some but not all of the clones sharing peptide specificity, regardless of the individual. There was a greater variation in the TCR α CDR3 sequences, where only the selection of a methionine residue as the first amino acid at the N-terminus of the CDR3 loop could be observed in three TCCs specific for epitope 3 (Fig. 3(A), XPAA 4 I, XPAS

(A)

93DTLEKISNEIKIVATP108

T cell clone	TCR gene segments	TCRAV	CDR3	TCRAJ	TCRAC
XPHC 2 III	V5S1J21C	GCAGACTCAGCTACCTACCTCTCTGCTCTC A D S A T Y L C A L	CGGGTATCACTTCACCAATTTTAC R V Y N E N K F Y	TTTGGATCTGGGACCAACTCAATGTAAACCA F G S G T K L N V K P	AATATCCAG N I Q
XPMS 2 I	V2S1J57C	AGTGATTCAGCCACCTACCTCTGTCGGTGAAC S D S A T Y L C A V N	ATTGTGGGTACTCAGGGCGGATCTGAAAGCTGGTC I V G T O G G S S E K L V	TTTGGAAAGGSAACCAACTGACACTAAACCCA F G K G T K L T V N P	TATATCCAG Y I Q
XPSZ 97 III	V18S1J15C	TCAGACTCAGCGGTACTCTCTGCTCTCTC S D S A V Y F C A L	TCAGCCCTCAACAGGAGGAACTGCTCTGATC S P L N Q A G T A L L I	TTTGGGAAGGGAACCACTTATCACTGAGTTTC F G K G T H L S V S S	AATATCCAG N I Q
XPTF 1 I	V8S1J44C	GAAGACTCGGCTGCTACTCTCTGCTCA E D S A V Y F C A	GCAAGTAGGAAGGACCTACTGCTCACTAACTCAC A S R K D R T A S K L L I	TTTGGACTGGAAACCAACTTCAGGTCAGCTC F G T G T R L Q V T L	GATATCCAG D I Q

(B)

93DTLEKISNEIKIVATP108

T cell clone	TCR gene segments	TCRBV	CDR3 (TCRBD)	TCRBJ	TCRBC
XPHC 2 III	V6S2/3D1J1S1C1	GGCGTGATCTCTGTGCCAGCAGC A V Y L C A S S	CCCTCAACAGGTCTCTGGGTGAAGCTTTC P Q Q V L G E A F	TTTGACAAGGCAACGACTACAGTTGTA F G Q G T R L T V V	GAGGACCTG E D L
XPMS 2 I	V12S2D1J1S2C1	TCGTGTACTCTCTGTGCCATC S V Y F C A I	AGAGAGGACAGAAATACTATGGCTACAC R E D R N Y Y G Y T	TTTGGTTCCGGGACAGGTTAAGCGTTGTA F G S G T R L T V V	GAGGACCTG E D L
XPSZ 97 III	V5S2/3D2J2S1C2	GGCCCTATCTCTGTGCCAGCAGC A L Y L C A S S	CCCGCGGGGGTCTCTACAAAGGAGCTTC P R G G S Y N E Q E	TTCGGGCCAGGACACGGCTACCGTGCTA F G P G T R L T V L	GAGGACCTG E D L
97 III	V5S2/3D2J2S1C2	GGCCCTATCTCTGTGCCAGCAGC A L Y L C A S S	CGGGCGCGGGTCTCTACAAAGGAGCTTC R G R G S Y N E Q E	TTCGGGCCAGGACACGGCTACCGTGCTA F G P G T R L T V L	GAGGACCTG E D L
XPTF 1 I	V5S1D2J2S3C2	GGCCCTTATCTTTGGCCAGCAGC A L Y L C A S S	AAGCGGACAGGGGTTTCCACAGATAGCCATAT K C T G S V S T R T Q Y	TTTGGCCAGGACACCGGCTACAGTCTCTC F G P G T R L T V L	GAGGACCTG E D L

Fig. 2. The TCR α (A) and TCR β (B) chain sequence analysis of Bet v 1 epitope 2 (aa 93–108)-reactive TCCs. These sequence data are available from EMBL/GenBank/DBJ under the accession numbers X92781–X92789. See legend to Fig. 1.

(A)

113ILKISNKYHTKGDH126

T cell clone	TCR gene segments	TCRAV	CDR3	TCRAJ	TCRAC
XPAA 4 I	V13S1J41C	GGAGACTCAGCCACCTACTCTCTGTGCAGGAGC G D S A T Y F C A A S	ATGGGGCTTAATCCGGGGTATGCACCTAAC M G L N S G Y A L N	TTGGGAAAGGCACTCTGCTGTGCACACC F G K G T S L L V C P	GATATCCAG D I Q
XPAS 16 I	V8S2J35C	GGAGACTCAGCTCTGTACTTTTGTGCAGAG G D S A V Y F C A E	AATAGGGGCTCTGGGTACTAGCTATGGAAGCTGACA N R C A G G T S Y G K L I	TTTGGACAAGGCACTCTTCACTGTCCATCCA F S Q G T I L C V H P	AATATCCAG N I Q
27 I	V8S2J11C	GGAGACTCAGCTCTGTACTTTTGTGCAGAG G D S A V Y F C A E	ATGATGAATCAGGATACAGCACTCTGAGC M M N S G Y S T L T	TTTGGAAAGGCACTATGCTTCTAGTCTCTCCA F S K G T M L L V S P	GATATCCAG D I Q
XPHC 33 III	V6S1J28C	GGGACTCAGCAATGTACTCTGTGTGCA G D S A M Y F C A	ATGAGACATCTTACTCTGGGGTGGGAGTTACCACTCACT M R C L Y S G A G S Y Q L T	TTGGGAAAGGCACTCACTCTCGGTATCCCA F G K G T K L S V I P	AATATCCAG N I Q
46 IV	V2S1J34C	AGTGATTCAGCCACCTACTCTGTGCGCGIG S D S A T Y L C A V	CCCTTCTATACACCGCAAGCTCATC P V Y N T D K L I	TTTGGGACTGGGACAGATTACAGCTTTTCCA F G T G T R L Q V F P	AATATCCAG N I Q
46 IV	V11S1J31C	GCAGATGCTGTGTTTACTACTGTGCTGTG A D A A V Y Y C A V	AGCAATACCAATCTAGACTCATG S S N N A R L M	TTTGGAGATGGAACCTCACTGTGTGGTGAAGCC F G D G T Q L V V K P	AATATCCAG N I Q

(B)

113ILKISNKYHTKGDH126

T cell clone	TCR gene segments	TCRBV	CDR3 (TCRBD)	TCRBJ	TCRBC
XPAA 4 I	V14S1D2J1S4C2	TCCTGTACTCTCTGTGCCAGCAGC S L Y F C A S S	CGGACTATGCAAAACTCTTTT R T N E K L F	TTTGGCAGTGGAAACCACTCTCTGTCTTTC F G S G T Q L S V L	GAGGACCTG E D L
XPAS 16 I	V6S2/3D2J2S7C2	GGCGTGATCTCTGTGCCAGCAGC A V Y L C A S S	TTAGCTACAGGACTCTCTACGAGCAGTAC L A R G T L Y E Q Y	TTGGGCGCGGACCAAGGCTACAGTCA F G P G T R L T V T	GAGGACCTG E D L
27 I	V13S5D1J1S1C1	TCCTGTACTCTCTGTGCCAGCAGT S V Y F C A S S	GAAGAACAGGGGGCTATGCGTTTC S E Q G A I A F	TTTGGACAAGGCAACGACTCACTGTGTA F G Q G T R L T V V	GAGGACCTG E D L
XPHC 33 III	V13S2D2J2S7C2	TCCTGTACTCTCTGTGCCAGCAGT S V Y F C A S S	CTCACACCGGCTGAAAGCAGTAC L T S G L K K Q Y	TTGGGCGCGGACCAAGGCTACAGTCA F G P G T R L T V T	GAGGACCTG E D L
46 IV	V13S3D1J1S2C1	TCCTGTACTCTCTGTGCCAGCAGT S V Y F C A S S	GAATCGGGGGTTTAGCGCTACAGC E S G G L G G Y T	TTGGGTTGGGACCAAGGTTAAGCGTTGTA F G S G T R L T V V	GAGGACCTG E D L

Fig. 3. The TCR α (A) and TCR β (B) chain sequence analysis of Bet v 1 epitope 3 (aa 113–126)-reactive TCCs. These sequence data are available from EMBL/GenBank/DBJ under the accession numbers X92875–X92885. See legend to Fig. 1.

27 I, XPHC 33 II). For epitope 1 specific TCRs, the motif LR was seen in three β CDR3 sequences at the N-terminal portion of the loop (Fig. 1(B), TCCs XPBP 53 I, XPGZ 17 I, XPHC 8 III). In a fourth sequence the L and R residues were separated by an A residue, but still located at the N-terminus (Fig. 1(B), XPSZ 11 II). Four out of five TCCs specific for epitope 3 revealed a G residue as the fourth amino acid in their β CDR3 sequences (Fig. 3(B), XPAS 16 I, XPAS 27 I, XPHC 33 III, XPHC 46 IV). Among these, the CDR3s of the β chains of three clones were rearranged to TCRBV13 family members (Fig. 3(B), XPAS 27 I, XPHC 33 III, XPHC 46 IV).

Promiscuous Bet v 1 peptide recognition mediated by identical TCRB chains

The TCCs XPSZ 11 II and XPAS 16 I expressed diverse TCRA chains, but they utilized identical TCRB chains. Both TCCs expressed TCRBV6S2/3 rearranged via BD2 to BJ2S7 and shared identical aa sequences in the CDR3. The TCRB chain nucleotide sequences of both clones were also identical, in spite of the fact that these TCCs recognized different peptides (Figs 1(B) and 3(B)).

Dual receptor T cells

Two signals for TCRAV families were obtained from RNAs two of the TCCs (Fig. 1(A): XPSZ 31 II; Fig. 3(A): XPHC 46 IV) when amplified with TCR α primers. One clone yielded two signals for TVCRBV families (Fig. 2(B): XPSZ 97 III). Sequence analysis of the PCR products showed that all were rearranged in frame and coded for functional α or β chains. In all cases the second chain of the heterodimer of the same clone was present only as a single rearrangement.

DISCUSSION

This study was designed to investigate the diversity of TCR usage by CD4⁺ T cells in atopic individuals sensitized to the major birch pollen allergen Bet v 1, in order to evaluate the potential of therapeutic strategies targeted at allergen-specific TCRs. We have analysed the TCR α and β chain expression by 16 TCCs from eight patients, which recognized three MHC class II restricted major epitopes of Bet v 1 (aa77–92, aa93–108, aa113–126). We have observed different TCR selection criteria for each epitope peptide studied.

The most obvious selection of sequence characteristics was found for the TCRs utilized by the seven TCCs specific for epitope 1 (aa77–92). Three out of seven TCCs used an AV8 family member. Two TCCs from one individual expressed an AV2 family member and the BV2S3 segment. Six of the seven TCCs rearranged either BJ1S4 or BJ2S7. TCRBJ1S4 is rarely used by T cells from peripheral blood, as opposed to the frequent use of BJ2S7 (Rosenberg *et al.*, 1992). This makes it very likely that BJ1S4 was positively selected for recognition of epitope 1. The length of the β chain CDR3 was conserved to from

nine to 10 amino acids. The α CDR3 showed no obvious conservation in length (ranging from eight to 15 amino acid residues) or in amino acid composition. Four of eight CDR3 sequences were from 12 to 15 amino acids long, which is above the average length found for CDR3 regions in T cells from peripheral blood (Moss and Bell, 1995). Although the TCRs specific for epitope 2 did not show conservation of particular motifs, their TCRB CDR3 loops revealed a fairly narrow distribution of lengths between nine and 11 amino acids (Table 2).

Epitope 3-specific TCRs exhibited a wider distribution in CDR3 lengths with the α chain loops ranging from eight to 14 amino acids and the β chain loops from seven to 10 (Table 2). Selection of TCRAV8 within an individual and selection of TCRBV13 between individuals was evident in the response to epitope 3. Whereas some V β families predominate the peripheral repertoire, others are only rarely used, such as TCRBV13 (Rosenberg *et al.*, 1992). This makes the selection of this family in TCCs specific for epitope 3 rather plausible.

The TCRAV8 appeared in six out of 16 TCCs in response to all three Bet v 1 epitopes. Although TCRAV8 belongs to the most frequently observed TCR α families (its frequency is generally around 10% in the α chain repertoire in peripheral blood; Robinson, 1992), it occurred with an increased frequency of 38% in the allergen specific TCCs. In this context, it is interesting to note that the TCRs of two out of five TCCs specific for a previously described minimal epitope (aa144–153) of Bet v 1, also used an AV8 segment (Breiteneder *et al.*, 1995). Moreover, Wedderburn *et al.* (1993) found that 50% of their Der f 1-specific TCCs (3/6) used AV8 segments.

The length of the TCRB CDR3 loops of all TCCs investigated here was distributed between seven and 11 amino acids. It is interesting to note that the TCRB CDR3 loops for another Bet v 1 epitope (aa144–153, ref. 31) were between six and 14 amino acids long. In this study, we also found that the TCR α chain sequences showed a greater variation in sequence characteristics when compared to the β chains. Selection of certain features was more obvious in the β chains. Therefore, it is tempting to speculate that the TCR β chain might be more involved in the interaction with the peptide than the TCR α chain. From the variety of sequences in the CDR3 regions of TCRs with the same epitope specificity, we also conclude that these junctional regions are likely to interact with the MHC II molecules as well.

The expression of two identical TCR β chains by two unrelated TCCs with specificity for different peptides and diverse α chains, was described by Giegerich *et al.* (1992) and termed promiscuous peptide recognition. Although the two TCCs described in that paper shared identical TCRB aa sequences, they differed by four nucleotides in the VDJ junctional regions. In contrast, we found that the β chains of our clones XPSZ 11 II (specific for aa77–92) and XPAS 16 I (specific for aa113–126) were not only identical on the aa level but also on the nucleotide level.

An absence of conservation of the junctional region was apparent in TCRs specific for two atopic allergens,

i.e. house dust mite total extract (Wedderburn *et al.*, 1993) and a defined epitope of Bet v 1 (Breiteneder *et al.*, 1995). These findings, which are consistent with our data on all three Bet v 1 epitopes, would imply interaction of the CDR3 loop not only with the antigenic peptide but also the MHC molecule.

In two TCCs (XPSZ 31 II, Fig. 1(A); XPHC 46 IV, Fig. 3(A)), two distinct in-frame TCRA sequences were detected. Sequencing of several independent β cDNAs of these clones did not reveal the presence of a second TCRBV. It has been shown that up to one-third of mature T cells express two TCRA chains as part of two functional and independent TCRs (Padovan *et al.*, 1993). One TCC (XPSZ 97 II, Fig. 2(B)) was found to have a second functionally rearranged β chain product. A second α chain was not detected. The two β chain sequences which were obtained from several independent pCRII clones only differed in the N-D-N portion of the CDR3 region. They were also of the same length (four aa residues). This indicates that the addition of D and N elements occurred on a single precursor product and produced two closely related TCR β chains judged by primary sequence standards. It cannot be stated which of the β chains was paired with the single α product for antigen recognition. The presence of a proline residue which significantly alters protein conformation makes it very unlikely that both β chains share the same specificity. It was shown that the expression of two β chains on close to 1% of human T cells is likely to be due to the escape of allelic exclusion (Davodeau *et al.*, 1995).

Each donor used in this study showed a distinct HLA-DR haplotype (Table 1). Therefore, it can be assumed that different HLA-DR alleles were used to present a given epitope peptide. The polypeptides encoded by each HLA-DR allele of a given individual are also involved in many different peptide-MHC complexes. The structurally less diverse CDR1 and -2 loops of TCRs are selected by the α -helical regions of the MHC molecule. Therefore, the most specific constraints on the TCR sequence are most likely imposed in the CDR3 region by the peptide bound to the MHC molecule.

Elucidation of the ways in which the TCR CDR3 interacts with the peptide-MHC class II complex will have important implications for understanding the function of the immune system, as well as for the treatment of immunologically mediated diseases. New concepts for immunotherapy will also be centered around the TCR. If a TCR-based therapeutical approach for atopic diseases is envisioned, it will have to take into account all peptides that are produced by processing a single allergen by the APCs. It will only be cost-effective if the peptide-specific populations of TCRs from different individuals can be targeted by a single agent, such as an antibody or a ligand. Any therapeutical approach that targets the TCR relies on its three-dimensional structure. Structural predictions made by computer modeling, based on the primary TCR sequences, are still unreliable. Definitive three-dimensional structures can only be obtained by X-ray crystallography. Even if the *in vivo* response to allergens is characterized by a limited TCR repertoire, it will

not be feasible to crystallize each distinct receptor. Therefore, only empirical data will tell whether peptide-specific TCRs of different primary sequences share three-dimensional structures.

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