

Conserved sequence motifs of CDR3 loops of TCR specific for two major epitopes of the grass pollen allergen Phl p 1

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Abstract

We analyzed the cDNA sequence data of complementarity determining regions (CDR3) of epitope mapped $\alpha\beta$ TCR of T cell clones (TCC). The TCC were specific for the major timothy grass (*Phleum pratense*) pollen allergen Phl p 1 and were derived from the peripheral blood of seven unrelated grass pollen-allergic individuals. Each TCR recognized one of two immunodominant T cell epitopes, PP73 or PP103, of Phl p 1. Although a diversity of recombined V and J segments was observed, amino acid motifs as long as five residues were conserved among CDR3 loops of TCR from TCC of different atopic individuals specific for the same peptide. The conserved sequences could comprise as much as 60% of the CDR3. All amino acid residues of the motifs of the CDR3 β and most of the CDR3 α of all TCR used in this study were encoded by randomly added nucleotides. This indicates that they were specifically selected for by the peptide bound to the MHC class II molecule. For one selected patient, a larger number of TCC, specific for PP103, was analyzed. The TCR repertoire was limited to three different TCR. The same MHC class II molecule, DRB1*1301, was identified to present PP103 to each of the three TCR.

Introduction

Type I allergy to pollen is a common disease in developed countries. In a recent multi-center study, where >30% of the study population were considered atopic, the highest rate for prevalence of skin sensitization was observed for grass pollen at 12.7% (1). The group I pollen allergens Phl p 1 from timothy grass, several isoforms of Lol p 1 from ryegrass and Sec c 1 from rye were characterized on a molecular level, and were found to share high amino acid sequence similarities (2–4). IgE antibodies specific for group I allergens of grass pollen are known to cross-react (5).

T lymphocytes play an important role in the induction of IgE synthesis, the crucial event in the development of type I allergy (6,7). T cell epitopes of Phl p 1 were identified by synthetic overlapping dodecapeptides (8). Some of these peptides were recognized by T cells clones of almost every *Phleum pratense* pollen allergic patient. The corresponding amino acid sequences of Phl p 1 were termed immunodominant epitopes. It was also shown that the allergen fragments were presented by human HLA-DR molecules.

Mature CD4⁺ T lymphocytes recognize allergenic peptides

presented by MHC class II molecules via their TCR (9). X-ray structures of individual TCR chains and $\alpha\beta$ TCR confirmed that the TCR contains Ig-like domains (10–12). Both the α and the β chains comprise three hypervariable loops, the complementarity determining regions (CDR) (13,14). The CDR1 and CDR2 in the V region mainly contact the polymorphic regions of the MHC class II molecule. The CDR3 in the junctional (NDNJ) region is positioned over the antigenic fragments placed in the groove of the MHC class II molecule (13).

Restriction of gene usage for both the TCR and the MHC molecules may be a crucial factor for triggering an aberrant immune response. T cell clones (TCC) derived from the synovial fluid of patients with rheumatoid arthritis (15) and TCC specific for a mycobacterial 65 kDa heat shock protein (16) showed conservation of either the V α or V β gene repertoire. Several investigators found that TCC specific for myelin basic protein (MBP) showed a non-random V gene usage (17, 18). In contrast, Meinel *et al.* (19) described a rather heterogeneous T cell response with respect to TCR gene

usage and HLA restriction, but a restricted epitope profile of MBP. In the context of type I allergy, investigations about epitope-mapped Bet v 1-specific TCC from birch pollen-allergic patients showed a rather diverse V gene usage (20), whereas Wedderburn *et al.* (21) described a limited TCR usage for house dust mite-specific TCC.

In two preceding papers from our group, CDR3 regions from TCR specific for several epitopes of Bet v 1, the major allergen of birch pollen, were analyzed (20,22). Looking for homologies at the DNA and protein level, no striking evidence was found. In consequence, we speculated in which way different primary sequences would still share three-dimensional structures. This led us to the concept of 'common sequence motifs' where we took into consideration that short amino acid motifs could lead to comparably folded CDR3 regions. These data would encourage investigations for TCR-based therapeutical approaches for atopic diseases in which TCR from different individuals could be targeted by a single agent.

In this study, we characterized TCR of Phl p 1 epitope-mapped TCC from grass pollen-allergic patients by molecular cloning and sequencing. The clones were established from peripheral blood and shown to be restricted by HLA-DR molecules. Amino acid sequence comparisons were done to identify conserved amino acids or even amino acid sequence motifs in the CDR3 regions where most of the peptide specificity would reside because of the junctional diversity of the V(D)J recombination (9).

Methods

Donor selection

Blood was obtained from seven grass pollen-allergic unrelated adult donors. All atopic individuals showed typical case histories, positive skin prick tests and their sera contained IgE that bound to Phl p 1 in immunoblots.

Phl p 1 and synthetic Phl p 1 peptides

Recombinant (r) Phl p 1 was expressed in *Escherichia coli* as a non-fusion protein, purified by ion-exchange chromatography and characterized by immunoblotting (M. Susani, unpublished results). A panel of 76 dodecapeptides overlapping for three residues was synthesized according to the Phl p 1 sequence by Cambridge Research Biochemicals (Cambridge, UK) and used for epitope mapping of TCC as described (8,23). The peptides were used at a concentration of 5 µg/ml in all experiments. The nomenclature for the peptides was: PP (for *Phleum pratense*) and the number of the first amino acid residue according to its original position in the Phl p 1 sequence. The sequences of the peptides recognized by the TCC investigated in this study were: PP73, amino acids 73–84: YHFDLSGHAFGA; PP103, amino acids 103–114: FEIKCTKPEACS.

Allergen-specific T cell lines (TCL) and epitope-specific TCC

Short term allergen-specific TCL were generated from peripheral blood mononuclear cells (PBMC) of grass pollen-allergic donors by stimulation with rPhl p 1 (5 µg/ml; 8). For TCC, T cell blasts were seeded in limiting dilution (0.3

cells/well) into 96-well round-bottom plates (Nuncclone; Nunc, Roskilde, Denmark) in the presence of 10^5 irradiated (5000 rad) allogenic PBMC as 'feeder cells', 1% phytohemagglutinin (PHA) v/v (Gibco, Grand Island, NY) and rIL-2 (4 U/well). Growing microcultures were then expanded weekly by turns of specific (Phl p 1) and unspecific (rIL-2) stimulation. The stimulation index was assessed by a standard [^{3} H]thymidine incorporation assay. (23). A 10-fold stimulation index was considered positive. Epitope mapping was performed as described (8).

Analysis of the phenotype of TCC

The surface markers of TCC were analyzed by flow cytometry, using a fluorescence-activated cell sorter (FACScan; Becton Dickinson, Mountain View, CA). For phenotype analyses, FITC-labeled mAb, anti-Leu 4/CD3, anti-Leu 3a/CD4, anti-Leu 2a/CD8, anti-TCR $\alpha\beta$ WT 31 and anti-TCR $\gamma\delta$ I (Becton Dickinson) were used (23).

HLA typing and restriction

HLA typing was performed as described previously (24). Murine mAb against human MHC class II determinants, L 243 (anti- HLA-DR; Ig2a), Leu 10 (anti-HLA-DQ; IgG1) and B7/21 (anti-HLA-DP; IgG1) were purchased from Becton Dickinson. Proliferation assays were performed in 96-well plates as described (25). TCC (2×10^4 cells/ml) were cultured with autologous irradiated PBMC (5×10^4 cells/ml) in the presence of rPhl p 1. The antibodies directed against framework antigens of HLA-D region molecules were used at a final concentration of 25 µg/ml. mAb were considered capable of blocking when only 50% or less antigen-dependent proliferation was observed.

In one selected case (patient NO) a larger number of TCC specific for epitope PP103 was analyzed. The MHC class II molecule presenting the peptide PP103 was determined for each different TCR identified. For this purpose proliferation assays using a panel of matched and mismatched PBMC of HLA-typed individuals were performed to reveal the restriction elements.

First-strand cDNA synthesis and PCR amplification

Total RNA was prepared from 1×10^6 cells by the single-step guanidiniumisothiocyanate-phenol-chloroform extraction method (26). Single-stranded cDNA synthesis was carried out on 1.2–1.8 µg of total RNA primed with oligo(dT) and avian myeloblastosis virus reverse transcriptase. PCR was performed by amplification of TCR α and β cDNA with oligonucleotide primers complementary to TCR V and C regions at a final concentration of 0.5 µM (TCR typing Amplimer kits; Clontech, Palo Alto, CA). Reactions were incubated in a Perkin-Elmer PE 2400 DNA thermal cycler (Perkin-Elmer, Norwalk, CT). Reaction mixtures were subjected to 25 cycles of the following conditions: denaturation at 93°C for 45 s, annealing at 55°C for 30 s and extension at 72°C for 30 s.

Cloning and sequencing of TCR α and β chains

The PCR reaction products were cloned into the TA cloning vector pCRII (Invitrogen, San Diego, CA) and sequenced by the Sanger dideoxy chain termination method (27) with

Table 1. Determination of the MHC class II phenotypes as described in Methods

Patient	HLA-DRB family	HLA-DQB family
EC	DRB1*07, DRB1*16; DRB4, DRB5	DQB1*01; DQB1*02
GZ	DRB1*07, DRB1*08; DRB4	DQB1*03; DQB1*04
HER	DRB1*01, DRB1*04; DRB4	DQB1*01; DQB1*03
KH	DRB1*11, DRB1*17; DRB3	DQB1*02; DQB1*03
LV	DRB1*04, DRB1*15; DRB4, DRB5	DQB1*01; DQB1*03
PS	DRB1*07, DRB1*15; DRB4, DRB5	DQB1*01; DQB1*02
NO	DRB1*0401, DRB1*1301; DRB3*0101, DRB4	DQB1*0603; DQB1*0302
GO*	DRB1*04, DRB4 (homozygous)	DQB1*03 (homozygous)
RO*	DRB1*04, DRB1*13; DRB3, DRB4	DQB1*01; DQB1*03

Patients are identified by their initials. An asterisk marks the non-allergic mother (RO) and father (GO) of donor NO. GO was homozygous, which made his B cells ideal candidates for usage as APC in proliferation inhibition experiments.

Sequenase version 2.0 (US Biochemicals, Cleveland, OH). For each of the TCR α and β chains studied, three independent pCRII clones were sequenced to identify possible sequence errors caused by nucleotide misincorporation by the Taq polymerase. Sequences were compared with gene databank entries (GenBank, Los Alamos, NM; EMBL, Heidelberg, Germany) and published TCRJ segments (28–33).

Results

HLA typing of the donors (Table 1)

Donors EC and PS had very similar haplotypes with only one difference (DRB1*16 or DRB1*15). These donors were not related to each other. Donors HER and NO shared DRB1*04 and DRB4. Subtyping revealed the subfamily 01 of DRB1*04 for donor NO, whereas for HER the subfamily was not determined. All other donors showed no significant similarities among their haplotypes.

TCC and epitope mapping (Table 2)

Twenty-five TCC derived from seven grass pollen-allergic individuals were included in this study. Eighteen clones were derived from one patient (NO). All clones had the helper cell phenotype CD3⁺CD4⁺ and expressed the $\alpha\beta$ TCR. Using the DR-specific antibody L243, which recognizes a determinant common to all DR molecules, proliferation in response to stimulation with rPhl p 1 could be blocked in all TCC in a dose-dependent manner. Stimulation could not be blocked by anti-DP and anti-DQ antibodies, indicating restriction by HLA-DR molecules. Each TCC recognized one of two immunodominant T cell epitopes of Phl p 1 (8). Peptide PP73 (amino acids 73–84) was recognized by five TCC from five patients, peptide PP103 (amino acids 103–114) was recognized by 20 TCC from four patients.

TCR α and β chain analysis of epitope-specific TCC

PP 73 (Fig. 1). Five TCC, each from a different patient, used five different TCR to recognize this peptide. Only two of these patients had similar HLA-haplotypes (EC and PS). The clones of these donors used different TCR to recognize PP73. Only

Table 2. V segment usage of epitope-mapped TCC

Phl p 1 peptide	Patient	TCC	TCRA/B
PP73	NO	V8S2/V17S1	
	EC	2 I	V8S1/V18S1
	GZ	53 I	V20S1/V13S1
	KH	27 I	V17S1/V17S1
	PS	8 I	V17S1/V1S1
PP103	NO	5 III, 9 III, 14 III, 18 III, 29 II, 36 III	V9S1/V13S1
		39 III, 42 III, 50 III, 58 III, 86 III, 16 III, 17 III, 29 III, 25 III, 57 III	V7S1/V4S1
		34 III	V1S1/V6S4
		29 I	V3S1/V5S1
	EC	29 I	V13S1/V20S1
	HER	47 I	V4S1/V6S3

The amino acid sequences of the T cell epitopes PP73 and PP103 are listed in Methods. TCRAV and TCRBV segments were assigned according to sequences previously published using the standardized TCR nomenclature (28–33).

TCC XPPS8 I and XPKH27 I, which differed in their BV gene usage (BV1S1 respectively BV17S1), used identical AV and AJ gene segments (AV17S1 and AJ50) in their α chains. The N nucleotides in their CDR3 regions differed from each other. Moreover, the HLA haplotypes of the donors of these clones (PS and KH) showed no similarity in their DRB families. The two clones XPNO32 III and XPEC2 I used AV8, but the first subfamily S2, and the other S1. They also differed in their TCRAJ segments (AJ54 and AJ49 respectively). The donors of these clones (NO and EC) had only the HLA haplotype DRB4 in common.

PP103 (Fig. 2). Seventeen TCC of 20 (85%) from the patient NO were stimulated by this peptide. They could be divided into three groups according to the V and J segments of their TCR: (i) 11 clones (64.7 %) used AV9S1/BV13S1, (ii) five clones (29.4%) used AV7S1/BV4S1 and (iii) one clone (5.9%) used AV1S1/BV6S4. Within each group, the CDR3 regions were identical. The other three TCC from three additional patients used different V families in comparison to NO but also to each other. Clone XPHER47 I also used BV6 as did XPNO34 III, but a different subfamily (S3 in contrast to S4 used by XPNO34 III) and a different BJ gene segment (S2 in contrast to S5 used by XPNO34).

Conserved sequence motifs in the CDR3 regions of TCR with the same epitope specificity

In general, there was a variation in lengths of the CDR3 regions that ranged from 9 to 12 amino acids for V α and 7 to 11 amino acids for V β . Despite the heterogeneity of V and J segments used, certain conserved amino acids or even conserved amino acid sequence motifs were identified in the CDR3 region.

All CDR3 α of TCC specific for PP73 had either a glycine or an alanine in position 1 (Fig. 1, top). Three out of five TCR had a basic residue, arginine or lysine, in position 3 of the CDR3 α . The amino acids in position 1 and 3 of the CDR3 α were encoded by randomly added nucleotides. Clones XPKH27 I and XPPS8 I which displayed the same AV and AJ

PP 73: ⁷³ FEIKCTKPEACS ⁸⁴			
T cell clone	TCRA gene segments	accession number	CDR3 α
XPNO 32 III	V8S2J54	Z77846	GGAAATAGGGGGGGACAGGGAGCCCAGAAGCTGGTA G N R G G O G A O K L V
XPEC 2 I	V8S1J49	Z77842	GCAATCCAGAAACACCGGTAACCAAGTTCTAT A I Q N T G N O F Y
XPGZ 53 I	V20S1J20	Z77844	GGTGGCGCTAACGACTACAAGCTCAGC G G A N D Y K L S
XPKH 27 I	V17S1J50	Z77848	GCAAGCAGGGCCTCCTACGACAAGGTGATA A S R A S Y D K V I
XPPS 8 I	V17S1J50	Z77850	GCGCTGAAAACCTCCTACGACAAGGTGATA A L K T S Y D K V I

PP73: ⁷³ FEIKCTKPEACS ⁸⁴			
T cell clone	TCRB gene segments	accession number	CDR3 β
XPNO 32 III	V17S1J1S1C1	Z77841	ACAGACAGGGTCGATACTGAAGCTTTC T D R V D T E A F
XPEC 2 I	V18S1J1S5C1	Z77843	GACAGTTTCAATCAGCCCCAGCAT D S F N O P O H
XPGZ 53 I	V13S1J1S2C1	Z77845	GAAAAGGGGAGGGGAGCTAACTATGGCTACACC E K G R G A N Y G Y T
XPKH 27 I	V17S1J2S3C2	Z77849	ACAACAGGAGGAGCGCAGTAT T T G G A Q Y
XPPS 8 I	V1S1J2S1C2	Z77851	CGAACAGGGAACGGGTACAATGAGCAGTTC R T G N G Y N E O F

Fig. 1. Nucleotide and deduced amino acid sequences of TCRA (top) and TCRB (bottom) CDR3 regions of independent TCC specific for Phl p 1 epitope PP 73 are listed. CDR3 region length was defined according to Rock *et al.* (14). Gene segments were assigned as in Table 1. The underlined CDR3 amino acids are encoded by the J segments. The motifs, as described in the text, are boxed. Note that all CDR3 α regions start with glycine or alanine and three CDR3 α regions (of XPNO32 III, XPKH27 I and XPPS8 I) have a basic amino acid in position 3. The CDR3 β of XPEC2 I and XPGZ53 I both have an acidic amino acid in position 1 and XPKH27 I and XPPS8 I share the amino acid threonine and glycine in positions 2 and 3 (conserved amino acids are shown in bold).

family and had different N nucleotides shared both these features in position 1 and 3. Clone XPEC2 I, derived from a donor with a comparable HLA haplotype to donor PS, had the polar amino acid glutamine in position 3 of the CDR3 α .

In the β chains of three of five TCR a motif consisting of

the three amino acids GXG or GXA was identified starting at position 3 of the CDR3 β (Fig. 1, bottom). These motifs which were encoded by randomly added nucleotides comprised between 27 and 43% of the CDR3 β . Clones XPPS8 I and XPKH2 I shared the same amino acids in positions 2 and 3

PP103: ¹⁰³ YHFDLSGHAFGA ¹¹⁴			
T cell clone	TCRA gene segments	accession number	CDR3 α
XPNO 5 III	V9S1J57	Z77827	TATACTCAGGGTGGATCTGAAAAGCTGGTC Y T O G G S E K L V
29 III	V7S1J29	Z81024	GTGATAGGGGGTTCAGGAAACACACCTCTTGTC V I G G S G N T P L V
34 III	V1S1J17	Z77830	ACCCGAGCTGCAGGCAACAAGCTAACT T R A A G N K L T
XPEC 29 I	V3S1J27	Z77832	GACGCATTGTATGCAGGCAATCAACC D A L Y A G K S T
XPLV 24 I	V13S1J24	Z77835	GTGGTAGGCGACAGCTGGGGGAAATTGCAG V V G D S W G K L Q
XPHER 47 I	V4S1J40	Z77837	GGTACTACCTCAGGAACCTACAAATACATC G T T S G T Y K Y I

PP103: ¹⁰³ YHFDLSGHAFGA ¹¹⁴			
T cell clone	TCRB gene segments	accession number	CDR3 β
XPNO 5 III	V13S1J2S1C2	Z81026	AGGGGTGGGGCCGGGCTCAATGAGCAGTTC R G G A G L N E Q F
29 III	V4S1J1S2C1	Z81025	TACGGGTGGGGTATGGCTACACC Y G L G Y G Y T
34 III	V6S4J2S5C2	Z77831	TTAGGGGGGGGGCGACCCAGTAC L G G G A T Q Y
XPEC 29 I	V5S1J1S1C1	Z77833	GCCGGGACCCCTAAGTTAGAAGCTTTTC A G T P K L E A F
XPLV 24 I	V20S1J2S3C2	Z77836	ACCTCAAGTGGGCGGGATACGCAGTAT T S S G R D T Q Y
XPHER 47 I	V6S3J2S2C2	Z77838	TTACCAAGGTGTTGCCGGGGAGCTGTTT L P G G A G E L F

Fig. 2. TCRA (top) and TCRB (bottom) chain sequence analysis of TCC specific for Phl p 1 epitope PP 103. See also legend to Table 2. In the CDR3 α region there was a predominance of the GX(X)S motif, as described in the text. In the CDR3 β regions, XPNO5 III and XPNO29 III, the motif was flanked by leucine on one end and glycine on the other. In XPHER47 I the motif was also flanked by glycine, whereas the leucine on the other side was separated from the motif by a proline (see amino acids shown in bold).

(threonine and glycine). Clones XPEC2 I and XPGZ53 I both had an acidic amino acid in position 1 of their CDR3 β , whereas clones XPEC2 I and XPPS8 I (derived from donors with fairly similar HLA haplotype) were contrasting in that XPPS8 I had a basic arginine in position 1.

The CDR3 α of the TCR specific for PP103 contained the 3 or 4 amino acid residue motif GXS or GXXS (X = T) in four of six receptors and in one instance the related motif AXY (Fig. 2, top). These motifs were encoded by randomly added nucleotides and germline derived J segments. In comparison

to clones recognizing PP73, glycine or alanine in position 1 was rather the exception (in clone XPHER47 I) than the rule.

The core motif GXG or GXA (X = A, L or G), which was already observed in CDR3 β regions of clones specific for PP73, was found in four of six CDR3 β of TCR specific for PP103. In two cases, NO5 III and NO29 III (Fig. 2, bottom), this core motif was flanked by leucine on one end and glycine or alanine on the other. Thereby the motif was extended to 5 amino acid residues in length comprising 50–62% of the total CDR3 region. In all cases the amino acids of the motifs were encoded by randomly added nucleotides. In a third TCR (XPHER47 I) the GXA motif was also flanked by a glycine which was encoded by the J segment. The leucine on the other side was separated from the motif by a proline (Fig. 2, bottom). In all clones, glycine was a frequently used amino acid in the N-terminal half of the CDR3 β region.

*DRB1*1301 presents the peptide PP103 to three different TCR of one patient*

Three different TCR were identified among the TCC of patient NO which recognized PP103. Each TCR consisted of a different recombination of V and J segments (Fig. 2). The MHC class II molecule responsible for the presentation of PP103 to each of these TCR was identified as DRB1*1301. In all seven selected TCC representing three different types of receptors, only the mother's (RO) antigen-presenting cells (APC) induced T cell stimulation when tested with rPhl p 1. Thus, the MHC class II molecules of the father (GO) could be excluded to be responsible for epitope presentation. DQ molecules could be excluded by the use of framework antibodies against DR, DP and DQ. DRB3 and DRB4 could be excluded by non-reacting of TCC with a panel of DRB3- and DRB4-positive, but DRB1*13-negative APC. The only remaining MHC molecule responsible for presentation was DRB1*1301. Therefore, the presenting DRB molecule could be identified to be the same for all seven clones.

Discussion

In this study, we have analyzed the sequence data of CDR3 regions from epitope-mapped TCR of Phl p 1-specific TCC. The TCC were derived from seven unrelated grass pollen-allergic individuals. Although we observed a diversity of V and J gene segments used, certain conserved amino acids or even amino acid motifs in the CDR3 loops could be discerned.

Diversity as well as restricted gene usage of TCR repertoires against a known antigen or in the context of a disease has already been the subject of previous publications. In a study about clonal dominance in a TCR repertoire against a HLA-DR3-restricted hsp65 peptide, the authors state that this specific MHC class II-peptide complex was able to select for TCR V genes and amino acids in the CDR3 region (34). On the other hand, conservations within the TCR usage was more generally found in human class I-restricted responses (35–37), probably due to the very strict length requirements for MHC class I-presented peptides.

The fact that the same MHC class II molecule, DRB1*1301, was used to present the peptide PP103 to each of the three different TCR emphasizes the significance of conserved

sequences in their CDR3 regions. It seems obvious that certain amino acids in the CDR3 regions of these three receptors (XPNO5 III, XPNO29 III and XPNO34 III) encoded by randomly added nucleotides were specifically selected for by peptide PP103. The recombined V and J segments differed in each case.

HLA typing of the other patients showed a rather diverse combination of MHC class II molecules expressed by each atopic individual. It seems very likely that the allergenic peptide is presented by different class II molecules in each instance. Since the TCR of EC and PS recognizing PP73 were different, the peptide might be presented by the only differing HLA molecules, DRB1*15 and DRB1*16 respectively.

We were able to show that certain amino acids were used in conserved positions and amino acid motifs were conserved among TCR with the same specificity from TCC of different patients. Noticeable, three of five clones found in the α chains of PP73-specific TCR had a basic amino acid in position 3 of the CDR3. A motif GX(X)S was found in four of six clones (66.7%) in the CDR3 α region of PP73-specific TCR. In a sequence analysis study of human $\alpha\beta$ TCR CDR3 regions, 10.5% of 318 TCR CDR3 α regions derived from four healthy donors and one sample of fetal cord blood showed an amino acid combination GXS (38). The increased rate of usage of GXS in PP103-specific TCR CDR3 α makes the selection of these motifs by allergenic peptides highly plausible.

The scheme of the motifs in the β chain is basically the same for PP73 as well as PP103-specific TCR (GXG and GGA), except that β chains of PP103-reactive TCR have conserved amino acids (glycine and leucine) flanking the motifs. So it may be speculated whether the α chains might be responsible for epitope fine specificity in the case of reactivity against Phl p 1. It is important to notice that the motifs in the β chains were exclusively encoded by randomly added nucleotides and maybe small portions of the D segments, but never by the germline-derived J segments (Table 2 and Fig. 1).

The role of the amino acids that compose the motifs (glycine and serine) was investigated by a study of Prochnicka-Chalufour *et al.* (39). Analysis of total amino acid usage within the CDR3 regions of 80 human TCR sequences showed an excess of asparagine, glycine and serine residues when compared with framework residues. Moss *et al.* (38) also reported an increasing contribution from glycine residues following position 3 of the CDR3 α region. In this study, it is stated that the predominance of glycine residues in the CDR3 loops was no surprise, given the ability of this amino acid to form type II turns and impart flexibility into loops. In human CDR3 β , the most striking feature is the major contribution from glycine residues, particularly encoded by the NDN region in the middle of the loop. This is due to the fact that the TCRBD gene segment can encode glycine residues in all three reading frames. In the study of Garcia *et al.* (12) the authors reported the observation that many glycine and other small residues like alanine were found at the apices of the CDR of the crystallized TCR. These residues were found in positions likely to be within the TCR-peptide-MHC interface. The absence of protruding side chains could allow the TCR to approach the peptide-MHC complex surface more closely.

A study of 54 clones from 15 multiple sclerosis patients

with specificity to MBP and three healthy individuals showing heterogeneity of TCR gene segments also points at common sequence elements in the CDR3 (40). These data suggest that these CDR3 with common sequence motifs might fold into similar three-dimensional conformations to enable the contact with the presented peptides.

Based on the model of Garcia (12), it seems very likely that the conserved amino acids and the epitope-related motif described here play a crucial role in peptide-TCR interaction. Therefore, these findings could contribute to a discussion about new ideas for immunotherapy implying that different primary sequences share three-dimensional structures. Eventually it might be possible to develop therapy forms that specifically target different TCR populations by one single agent.

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Abbreviations

CD	cluster of differentiation
CDR	complementarity determining region
Lol p 1	major ryegrass pollen allergen
MBP	myelin basic protein
PBMC	peripheral blood mononuclear cells
PHA	phytohemagglutinin
Phl p 1	major timothy grass pollen allergen
rPHL p 1	recombinant Phl p 1
Sec c 1	major rye pollen allergen
TCC	T cell clone
TCL	T cell line

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