TCRB junctional regions from HLA-B27-restricted T cells and HLA-B27 binding peptides display conserved hydropathy profiles in the absence of primary sequence homology

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Abstract
Analysis of formal amino acid sequence identity between different TCRB chain (TCRB) hypervariable regions (CDR3) is commonly used to localize relevant sites of TCR antigen interaction or to yield indirect information on unknown corresponding antigens. However, this analysis sometimes fails to demonstrate expected concordances, e.g. between CDR3 from T cell clones of identical reactivity. Since this may be due to ignorance of physico-chemical parameters, we have now used hydropathy profile analysis as an additional method to examine TCRB-CDR3 and putative peptide antigens. Superimposed hydropathy plots (SHOP) of 20 TCRB-CDR3 from HLA-B27-restricted autoreactive and Yersinia enterocolitica-specific synovial cytotoxic T lymphocytes (CTL) isolated from patients with reactive arthritis (ReA) revealed restricted distribution of polar amino acids resulting in characteristically different SHOP profiles between the two CTL groups. Similarly, Yersinia-derived and self nonapeptides known to bind HLA-B27 differed in SHOP profiles. To validate the method we have extended SHOP analysis to published TCRB sequence data from additional HLA-B27- and HLA-A2-restricted CTL. Limited variability of hydropathy was observed in TCRB-CDR3 from peptide-specific CTL but not in TCRB from HLA-B27-alloreactive CTL or non-HLA-B27-restricted control CTL. We here demonstrate that SHOP may improve TCR-CDR3 sequence analysis by detection of structural constraints which remain cryptic by conventional sequence analysis. Our data suggest that electrostatic properties rather than rigid sequence motifs determine T cell specificities.

Introduction
Specificity of T cells for antigenic peptides presented on MHC molecules is mediated to a high degree by the complementarity determining regions (CDR3), i.e. the third TCRA and TCRB hypervariable sites (1). This critical function of the CDR3 in T cell specificity is suggested by the structural homology of the TCR-CDR3 to corresponding sites known to mediate specificity in Ig molecules (2,3), restricted utilization of amino acid residues in TCR-CDR3 of T cells with identical specificity (4-6) and studies using site-directed TCR mutagenesis (7) or chimeric TCR molecules (8). Further, elicitation of reciprocal charges in TCR junctional sequences (CDR3) after amino acid substitution at defined peptide positions (9,10) and altered antigen recognition after TCRB-VDJ junctional region mutagenesis have provided additional evidence that CDR3 are directly involved in peptide recognition (4,11). This prominent function of the CDR3 makes it an ideal target for specific immune intervention, especially in situations where detailed information on its interaction with MHC and peptide is available.

In the case of HLA-B27-restricted immune responses, a
large body of data on HLA-B27 structure and binding peptides has recently been published. Elucidation of the crystal structure of the HLA-B27 antigen (12,13) has shown that its conformation and its peptide-presenting properties are similar to other MHC class I molecules (14). Nonameric peptides are bound in extended conformation and anchored at both ends by peptide termini and residue 2 (12,15). These data are consistent with HLA-B27 peptide binding assays (16-19) and peptide elution studies. The majority of naturally processed self and viral peptides eluted from HLA-B27 molecules (20) were of nine amino acids length and consitutively shared an arginine at position P2, obviously essential to anchor the peptide in the ‘B45 pocket’ of the HLA-B27 molecule (21,22). Whereas the peptide termini probably contact the MHC molecule, the central part of the HLA-B27 bound peptide forms an exposed loop or bridge (13) and might therefore be accessible for the TCR. This implies that the peptide specificity of T cells is mainly directed towards the four to six core amino acid peptide residues which bulge out of the groove (23,24). TCR regions that are relevant for the contact with the peptide are thus expected to be concentrated to a small number of residues in the CDR3 loop.

From a clinical perspective, the MHC class I allele HLA-B27 confers susceptibility to certain rheumatic diseases such as ankylosing spondylitis, Reiter’s syndrome (RS) and other ‘seronegative spondylarthropathies’. This association and the fact that RS and reactive arthritis (ReA) are often preceded by infections with Gram-negative bacteria such as Yersinia, Salmonella, Shigella and Chlamydia may be caused by the T cell-mediated cross-recognition of structurally related bacterial and self antigens presented by the HLA-B27 molecule (25). In this scenario, it is hypothesized that tolerance to HLA-B27-presented ‘cryptic’ self peptides is abrogated through the activation of cytotoxic T lymphocytes (CTL) by bacterial peptides presented on HLA-B27 (26).

A better understanding of the interacting structures within the complex of MHC, peptide and TCR may help to test the above hypothesis and enhance the outlook for potential immune intervention in HLA-B27-associated diseases. We thus have previously analyzed the TCR repertoire of HLA-B27-restricted CTL specific for bacterial or self antigen and non-HLA-B27-restricted control CTL isolated from the synovial fluids of patients with ReA (27). Predominant usage of three highly homologous TCRBV families (Vβ13, 14 and 17) by HLA-B27-restricted CTL indicated that these TCRBV families share a preferred site for contact with the HLA-B27 molecule. However, only limited concordance was found within the putative peptide-contacting TCRBV-CDR3 regions. The failure to find more striking TCRβ-CDR3 homologies using formal amino acid alignment led us to address the question of whether we would observe a higher degree of CDR3 homogeneity at the physico-chemical level. A requirement for physico-chemical similarities (allowing charge-charge interactions, hydrogen bonds, hydrophobic interactions) between CDR3 and peptide is suggested by several studies which imply that TCR-CDR3 and peptide are attached in a longitudinal manner (12,13,15,28). In the present study, using TCRβ-CDR3 sequences from HLA-B27-restricted CTL, various control CTL and HLA-B27 binding peptides, we therefore introduce the comparative analysis of hydropathy profiles as an additional method to analyze TCR.

Methods

Patients

T cells were derived from synovial fluids of three HLA-B27-positive patients with ReA (one Yersinia enterocolitica-induced, one Chlamydia trachomatis-induced and one Salmonella-induced) and peripheral blood mononuclear cells (PBMC) of one healthy HLA-B27-positive donor as reported previously (27).

Clones and lines from CTL

CTL clones and lines were generated as previously described (29). In brief, cell lines derived from synovial fluid mononuclear cells (SFMC) or PBMC were propagated by stimulation with Y. enterocolitica-infected murine L cells, transfected with the HLA-B27 gene and the human β2-microglobulin gene (30), or C1R-B27 cells. T cell clones (Tc) were cloned from CD8+

enriched SFMC/PBMC or from T cell lines by limiting dilution. The functional characteristics of the resulting T cell clones were determined by standard 51Cr-release assay using autologous/allogeneic, infected/non-infected and TAP+/TAP− target cells which expressed HLA-B27 or alternative class I and class II MHC molecules. In addition, blocking assays were performed with antibodies specific for HLA-B27 (Me-1) (31), MHC class I and II molecules, TCR β and TCR γδ (27,29). By this method Y. enterocolitica-specific as well as auto-reactive CTL could be identified and propagated. Cytotoxic activity of HLA-B27-restricted CTL could be blocked by Me-1. For the remaining non-HLA-B27-restricted CD8+ CTL clones, the antigen specificities and restriction elements were not defined. These clones were designated non-HLA-B27-restricted control CTL.

Sequence analysis

RNA isolation from CTL clones, reverse transcription of mRNA, TCRBV-specific PCR and sequencing of the resulting amplification products were performed as published previously (27). The 3' ends of sequenced TCRBV genes were identified according to established criteria (32). A database search in EMBL and GeneBank DNA libraries was performed to verify TCRBV family and subfamily identity. True clones (CTLc) characterized by a single TCRBV transcript and a small number of CTL with two TCRBV transcripts were identified (27). Since it was impossible to subclone the latter, it cannot be decided whether these CTL are two-clonal lines (marked 2c) or clones expressing two TCRBV chains (33,34).

Definition of TCRβ-CDR3

For generation of hydropathy plots (HOP), CDR3 were defined to reach from an N-terminal constant cysteine at TCRB position 89–92 (32) to a C-terminal constant phenylalanine (F) preceding a GXG motif within the Jα element (G is glycine, X is any amino acid). By that definition, CDR3 include germine encoded sequences flanking the hypervariable N–D–N-joining region. For determination of CDR3 length, the first four amino acids, frequently encoded by the TCRBV gene, were excluded.
Limited hydropathy in HLA-B27-restricted TCRβ-CD3

Generation of HOP and superimposed hydropathy plots (SHOP)

To generate CDR3 HOP, hydropathy values were assigned to each amino acid according to the scaling of Hopp and Woods (35). This rating characterizes hydrophobic residues by hydropathy values < -1.3 whereas neutral amino acids range from -1 to +0.3. Hydropathy values of +3 indicate charged amino acids. All CDR3 sequences were adjusted to a constant cysteine (P1) by the definition given above. For comparative analysis of HOP from CDR3 or peptide sequences, HOP of defined CTL and peptide groups were superimposed (SHOP) for better detection of conserved motifs. Gaps or shifts to improve congruity between different CDR3 were not allowed or, in one case, explicitly stated in the text.

SHOP from published sequence data

To further validate the method, additional SHOP were generated from published TCRβ-CDR3 sequences of CTL with known fine specificity. We analyzed 25 TCR from HLA-A0201-restricted CTL specific for influenza matrix protein (MP) 58-66 (39), eight TCR from HLA-B27-restricted CTL specific for influenza NP383-391 (40) and 16 TCR from HLA-B27-alloreactive CTL (41,42). Furthermore, 11 self peptides eluted from HLA-B27 molecules (20), seven Y. enterocolitica-derived peptides (17) and the influenza nucleoprotein-derived peptide 383-391 (43), all known to bind to HLA-B27 molecules, were analyzed as putative CDR3 ligands.

Statistical analysis

When TCRβ-CDR3 SHOP analysis indicated limited amino acid usage at specific positions, respective sites were further statistically analyzed. Distribution of hydrophobicity values was tested for normality. If applicable, t-test analysis was performed.

Results

CDR3 lengths

In the present study, we analyzed TCRβ-CDR3 from four groups of HLA-B27-restricted CTL, i.e. CTL specific for Y. enterocolitica, self antigen (27), influenza A NP383-391 (40) and HLA-B27-alloreactive CTL (41,42). Furthermore we studied HLA-A0201-restricted CTL specific for influenza A MP58-66 (39) and non-HLA-B27-restricted control CTL of unknown restriction and peptide specificity (27).

CDR3 lengths were similar for all CTL except the HLA-A2-restricted group (mean number of amino acid residues ± SD; HLA-B27-autoreactive CTL: 11.2 ± 1.37, HLA-B27-Yersinia-specific CTL: 10.7 ± 1.21, non-HLA-B27-restricted controls: 10.9 ± 1.88, HLA-B27-alloreactive CTL: 10.8 ± 2.2, HLA-B27 influenza NP-specific CTL: 10.6 ± 0.52, HLA-A0201 influenza MP-specific CTL 8.8 ± 0.38).

Generation of superimposed hydropathy profiles from TCRβ-CDR3 regions (SHOP)

As reported previously (27), conventional amino acid sequence alignment of TCRβ-CDR3 regions from Y. enterocolitica-specific and autoreactive HLA-B27-restricted CTL which had been isolated from patients with ReA only revealed limited formal sequence homology. To investigate whether these CDR3 share physico-chemical properties, we now assigned hydropathy values to each amino acid according

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**Fig. 1.** SHOP from TCRβ-CDR3 of (a) 23 HLA-A0201-restricted CTL specific for influenza MP58-66 (39), (b) eight HLA-B27-restricted CTL specific for influenza NP383-391 (40) and (c) 16 HLA-B27-alloreactive CTL (41,42). Positions of restricted hydropathy are indicated by arrows. [This figure displays sequences from clones (a) JWZE11; MB6; JNBJ12; MODG2; KEF; BDE8; SWA13; SWA10; MBP3; MBP7; MPB8; SWA20; KDA24; KLE5; NMY12; MPB5; CLH5; JNBU12; BDE20; MODG2; SWA3; SAW9; SAW18; CDE10; HFI; SD16; SD13; SD2; (b) GM7; 28; 172DRF; 139DRD; 64.8P; G36; 102DRF; 64DRF; 67DRF; 212DRD; 40; 2DLE; 17A2; 168DRF; 22DAG; 5A2.]
Limited hydropathy in HLA-B27-restricted TCRB-CD3

to the scaling of Hopp and Woods (38). Using these data, we
generated HOP from TCRB-CDR3 of CTL clones (27,40) or
previously reported sequences of HLA-B27 binding peptides
(17,20,43). These HOP were then superimposed (SHOP) to
allow easier identification of physico-chemical concordances
within defined CTL and peptide groups (Figs 1–4).

It should be noted that P1–4 of most CDR3 are encoded
by 3' ends of TCRBV germline segments which frequently
code for Cys-Ala-Ser-Ser. Therefore, increasing hetero-
geneity of hydropathy between CDR3 from different CTL
clones is expected to begin at P5, which in most CDR3
represents the first hypervariable position.

HLA-A0201-restricted peptide specific CTL

to examine whether CDR3 from CTL specific for identical
peptide-MHC complexes share physico-chemical homo-
logsies, we generated SHOP from a panel of published HLA-
A0201-restricted V\textsubscript{B}17\textsuperscript{+} TCRB that interact with influenza A
MP 58-66 (39). As previously emphasized by Lehner et al.
(39), these TCRB-CDR3 display a high degree of sequence
uniformity, in particular when identical J\textsubscript{B} segments were
used. However, irrespective of the neighboring J\textsubscript{B} segments,
we found that the distribution of hydropathy was remarkably
similar in 23 of 25 TCRB-CDR3 with unique primary sequences
(Fig. 1a). These concordances were notably found at recombi-
national hot spots P5–8, were donor independent and did
not match four control CDR3 of HLA-A0201-restricted V\textsubscript{B}17\textsuperscript{+}
TCRB specific for other peptides (data not shown).

HLA-B27-restricted peptide specific CTL

The only known peptide-specific TCR restricted by HLA-B27
recognize influenza A NP383–391(40). Compared to the HLA-
A0201-restricted CTL, their TCRB-CDR3 SHOP diagram indi-
cates less extensive but still recognizable limitations, e.g. at
P8, where charged amino acids were absent. In addition,
there is a lack of hydrophobic residues at P9–11 (Fig. 1b). In
contrast to the HLA-B27-restricted autoreactive subset, which
typically used hydrophobic amino acid at CDR3 P5, influenza
A peptide-specific CDR3 were exclusively neutral at this
position.

HLA-B27-restricted alloreactive CTL

As a third independent group, we analyzed 16 TCRB from
HLA-B27-restricted alloreactive CTL (41,42). Although these
TCRB preferentially rearranged a limited number of related
V\textsubscript{B} segments, their TCRB-CDR3 SHOP diagram showed
outstanding variability (Fig. 1c).

HLA-B27-restricted autoreactive CTL

SHOP generated from 14 TCRB-CDR3 of autoreactive CTL
(Fig. 2a) demonstrated that in this group, neutral or hydro-
phobic residues represent the majority of amino acids, at P5–
9. Charged amino acids, defined by a hydropathy value of
+3, in contrast, were clearly under-represented at P5–9, and
totally absent from P5, P8 and P9 (indicated by arrows in
Fig. 2a).

HLA-B27-restricted Y. enterocolitica-specific CTL

At P5–9, TCRB-CDR3 from six Y. enterocolitica-specific CTL
(Fig. 2b) used neutral, hydrophobic or hydrophiiliic amino
acids at each individual position. At P9–11, hydrophobic
amino acids were completely absent from TCRB-CDR3 of
Y. enterocolitica-specific CTL (indicated by arrows in Fig. 2b)
but not from TCRB-CDR3 of autoreactive CTL and non-HLA-
B27-restricted control CTL (Fig. 2a and c).
Limited hydropathy in HLA-B27-restricted TCRB-CD3

Fig. 3. (a) SHOP of TCRB-CDR3 from a subgroup of HLA-B27-restricted autoreactive CTL. [CTL were derived from synovial fluid of three different ReA patients and from peripheral blood of one healthy donor and include clones P1.4.52; P6.2.22,2c1; P3.1.8; P6.2.36; P6.3.68; H1.1.7; P6.2.11; and P6.2.22,2c2 published by Duchmann et al. (27).] (b) TCRB-CDR3 from HLA-B27-restricted autoreactive CTL P6.2.22,2c1 and P3.1.8 differ in amino acid usage in the N-D-N-joining region but show almost identical hydropathy plots. [CTL were derived from synovial fluid of two different ReA patients (27).]

Non-HLA-B27-restricted control CTL
SHOP from 14 TCRB-CDR3 of non-HLA-B27-restricted control CTL showed a very heterogeneous distribution of hydropathy (Fig. 2c). There was, however, a preferential usage of hydrophobic amino acids at P5 and neutral amino acids at P7 by more detailed analysis.

Distribution of hydropathy at P5–9 and P9–11
Since SHOP of TCRB-CDR3 from HLA-B27-restricted autoreactive and Y. enterocolitica-specific CTL appeared to be different at P5–9 and P9–11, a separate analysis of these two regions was performed (Table 1). In CDR3 of non-HLA-B27-restricted control CTL, the frequency of hydrophilic, hydrophobic and neutral amino acids at P5–9 and P9–11 was remarkably consistent with the proportion of hydrophilic, hydrophobic and neutral residues in the pool of proteinogenic amino acids. This heterogeneous amino acid usage in the control group was clearly different from the distribution found in TCRB–CDR3 of HLA-B27-restricted autoreactive CTL (7.1% charged amino acids at P5–9) and HLA-B27-restricted CTL specific for Y. enterocolitica (0% hydrophobic amino acids at P9–11). Distribution of hydropathy at P5–9 of autoreactive CTL compared to control CTL was significantly different ($P = 0.024$, t-test).
Subgroups of HLA-B27-restricted autoreactive CTL

Analysis of a subgroup of HLA-B27-restricted autoreactive CTL demonstrated that seven out of 14 TCRB-CDR3 [or eight out of 14 TCRB-CDR3, if the sequence from clone P6.2.36 is shifted one position (P1 = P0) to achieve congruity] shared very similar hydropathy profiles spanning the first N-region diversity (Fig. 3a). Within each of these TCRB-CDR3, a hydrophobic residue was used at P5, hydrophilic amino acids were completely absent at P1–9 and all CDR3 used a neutral amino acid at P6. Predominant usage of hydrophobic or neutral amino acids at P5 was also present in a subgroup of non-HLA-B27-restricted control CTL (seven out of 14) but, in contrast to autoreactive CTL, similarities did not extend to P6–9. Furthermore, we identified two CDR3 from autoreactive CTL with almost identical HOP (Fig. 3b). These two clones had been derived from two different patients and differed substantially in their primary N-D-N region sequence. Pairwise comparisons of TCRB-CDR3 from bacteria-specific and autoreactive CTL did not reveal striking HOP concordances.

Peptides presented by HLA-B27

The observation that physico-chemical qualities of TCRB-CDR3 from HLA-B27-restricted CTL of different specificity were conserved and distinctly different suggested that these differences mirror properties of the putative natural CDR3 ligands, i.e. HLA-B27-presented peptides. To further evaluate this question, we generated SHOP from naturally processed nonapeptides eluted from HLA-B27 molecules of non-infected cells (20) and from HLA-B27 binding Yersinia-derived peptides (17). As illustrated in Fig. 4(a), self peptides eluted from HLA-B27 molecules (20) exclusively used a hydrophilic residue (arginine) at P2. This was expected, since the arginine P2 anchor is required for effective binding to the HLA-B27 molecule (12,20). Additional sites of restricted hydropathy were identified at P3, P5 and P6, i.e. positions that are not constitutionally involved in peptide binding to the MHC but are potentially accessible to the TCR. At P3 hydrophobic amino acids strongly predominated since charges were absent and only one out of 11 peptides used a neutral amino acid. No hydrophobic amino acid were found at P5 and hydrophilic amino acids were absent at P6 (arrows in Fig. 4a).

Although the absolute number of bacterial peptides analyzed was smaller, HLA-B27 binding peptides from Y. enterocolitica generally preferred usage of neutral and charged amino acids. Clearly, HLA-B27 binding Yersinia-derived peptides did not share characteristics of HLA-B27 binding self peptides since none out of five amino acids at P3 were hydrophobic and four out of five amino acids at P6 were charged (arrows in Fig. 4b). Since we had previously reported TCRB-CDR3 sequence homologies between a HLA-B27-restricted autoreactive CTL (27) and a HLA-B27-restricted CTL specific for influenza A NP383-391 (40) we also compared HOP from self peptides and NP383-391. In spite of a highly heterogenous primary sequence, there was a striking parallelism of the plot courses from the influenza peptide NP383-391 and one of the self peptides (Fig. 4c).

Discussion

In recent years, formal TCR sequence analyses with defined T cell clones have often been performed to obtain information on structural constraints of the TCR that result from its interaction with the MHC-peptide complex. Although these analyses provide only indirect evidence, they have often been the best information available, awaiting forthcoming functional systems defining T cell peptide specificity, amino acid substitution studies or the avenue of X-ray crystallographic to confirm their results.

In the present study, we introduce the analysis of distribution of hydropathy in TCRB-CDR3 by SHOP for the analysis of TCR. Applying this approach to different groups of HLA-B27, non-HLA-B27 and HLA-A2-restricted CTL, we demonstrate that SHOP analysis may detect characteristic physicochemical constraints in TCRB-CDR3 and MHC class I binding peptides which remain cryptic by conventional sequence analysis but may be biologically relevant.

In a former study, we have analyzed the TCRB chains of a large panel of HLA-B27-restricted autoreactive and Y. enterocolitica-specific CTL, which had been isolated from peripheral blood or synovial fluid of patients with reactive arthritis (27,29). Similar to HLA-B27-alloreactive CTL (41,42), we found that these CTL had preferentially rearranged a limited set of Vβ segments from three closely related Vβ families. Selection of these Vβ segments was independent of CTL peptide specificity, suggesting a common HLA-B27 binding motif. Consecutive formal sequence analysis of all TCRB-CDR3 sequences

Table 1. Distribution of hydrophilic, neutral and hydrophobic residues in the TCRB-CDR3 sites P5–9 and P9–11 of HLA-B27-restricted and non-HLA-B27-restricted control CTL

<table>
<thead>
<tr>
<th>P5–9</th>
<th>HLA-B27-restricted, autoreactive</th>
<th>HLA-B27-restricted, Yersinia-specific</th>
<th>non-HLA-B27-restricted, controls</th>
<th>proteinogenic amino acids</th>
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<tr>
<td></td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
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<tr>
<td>hydrophilic</td>
<td>5 (7)</td>
<td>10 (33)</td>
<td>17 (24)</td>
<td>4 (20)</td>
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<td>neutral</td>
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<td>14 (47)</td>
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<td>6 (20)</td>
<td>18 (26)</td>
<td>7 (35)</td>
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<td>P9–11</td>
<td></td>
<td></td>
<td>n (%)</td>
<td>n (%)</td>
</tr>
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<td>7 (39)</td>
<td>8 (19)</td>
<td>4 (20)</td>
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<td>0 (0)</td>
<td>12 (29)</td>
<td>7 (35)</td>
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</table>
demonstrated limited amino acid homologies in a small number of CTL. Based on the assumption that conservations in CDR3 of TCRB with identical MHC restriction might reflect properties of the antigenic peptide(s), this finding was compatible with the hypothesis that a limited set of bacterial or self peptides might be recognized by these CTL.

Since experiments from other groups (44) suggested that conventional sequence analysis alone does not exploit the full potential of TCR-CDR3 sequence information, we have now extensively reanalyzed these and additional groups of CTL and performed a detailed analysis of the distribution of hydropathy in TCRB-CDR3. Among the different systems rating amino acid hydropathy, we have used the scaling advocated by Hop and Woods (38), as this algorithm yields results that are in good agreement with alternative scalings (45) (data not shown) but separates polar (hydrophilic) from neutral and aromatic (hydrophobic) residues more clearly. In addition, the definition of the first position of TCRB-CDR3 obviously affects HOP analysis. We and others (35) have assigned position 1 (P1) of the TCRB-CDR3 sequences to a constant cysteine (Cys) which occurs near the C-terminus of all known Vβ segments. This definition is therefore based on the assumption that the high conservation and the covalent bondage quality of this Cys residue indicates a constant and important function in all TCRBV tertiary structures (32,46).

Independent of this rational, an alternative adjustment according to the residues' natural positions in the TCR B chain (32) was tested and yielded similar SHOP.

Although HOP are frequently used to characterize antigenic determinants or to predict transmembrane regions of integral proteins (47), generation of HOP and SHOP for the analysis of TCRB-CDR3 is novel. It is based on the assumption that polarity as an important parameter for molecular interactions also influences the association of the TCR to the MHC–peptide complex. That this is indeed the case was impressively shown by Jorgensen et al., who demonstrated that experimental charge inversion in peptide antigens leads to corresponding charge inversion in TRCA and TCRB-CDR3 (9). The importance of polarity in TCR–antigen interaction was further substantiated by the finding that characteristics of unknown CTL epitopes could be successfully predicted by focussing on charge potentials in TCRB-CDR3 (48–50). Other experiments showed that molecular mimicry in a MHC class II context depended on similar antigenic surfaces available for the TCR rather than amino acid sequence homology (44).

To test the validity of SHOP for the analysis of TCRB-CDR3 sequences, we examined a total of five different groups of CTL with known HLA restriction and antigen specificity, including two groups of CTL with known peptide specificity. Another group of non-HLA-B27-restricted CTL with unknown MHC restriction and unknown antigen specificity were used as unselected controls. In a first analysis HOP were generated from a large group of published Vβ17+ TCRB sequences from HLA-A0201-restricted, influenza A MP58–66-specific CTL (39). HOP from these TCRB-CDR3 showed remarkable concordances in the distribution of TCRB-CDR3 hydropathy. These concordances were donor independent, based on hydropathic properties of amino acids at specific positions rather than sequence identities and did not match four control CDR3 of HLA-A0201-restricted Vβ17+ TCRB-specific for other peptides. These results suggested that in this group of CTL, the peptide strongly selected appropriate TCRB–CDR3 in a direct or indirect manner. It also supported the hypothesis that TCRB-CDR3 from CTL recognizing similar peptide–MHC complexes displayed homologous hydropathy profiles. This was further substantiated by the finding that another group of peptide specific CTL, i.e. HLA-B27-restricted CTL specific for the influenza A NP383–391 (40) also showed restricted SHOP. We then tested the inverse hypothesis, i.e. that TCRB-CDR3 from CTL recognizing different peptide–MHC complexes display heterologous hydropathy profiles. Analyzing HLA-B27 alloreactive CTL, which are expected to recognize a large number of different epitopes (41), we found that the distribution of hydropathy among their TCRB-CDR3 was indeed completely random. A similarly heterogeneous SHOP profile was found among unselected non-HLA-B27-restricted control CTL, except at P5 and P7. Over-representation of hydrophobic amino acid at P5 seems to be a common feature of many TCRB-CDR3 and has already been reported by Moss et al. (35) who analyzed 161 TCRB-CDR3 from adult and fetal cord blood. Probably caused by Dβ gene segments which encode glycine (G) in all open reading frames, an excess of neutral residues at P7 is also seen in many TCRB-CDR3 (51, 52). Notably, in TCRB-CDR3 from HLA-B27-restricted autoreactive CTL, G was detected only once at this position but predominated at P6 (six out of 14, 42%).

After these analyses of SHOP of defined groups of TCRB-CDR3 yielded results that confirm reasonable predictions we used this method to reanalyze the TCRB-CDR3 sequences from HLA-B27-restricted CTL specific for self-antigens and Y. enterocolitica. In both groups of CTL, SHOP analysis demonstrated a limited and characteristically dissimilar distribution of TCRB-CDR3. These restrictions in TCRB-CDR3 hydropathy profiles were not specific for a given individual since autoreactive CTL were derived from SFMC/PBMC of three unrelated patients and one healthy individual. In contrast, non-HLA-B27-restricted control CTL, which had been isolated from the synovial fluid of one individual (P6), displayed a highly heterogenous SHOP profile. HOP restrictions in the TCRB-CDR3 of autoreactive CTL are also unlikely to be generated by chance since they span recombinational hot spots. In addition, HOP restrictions among TCRB-CDR3 from the HLA-B27-restricted autoreactive and Yersinia-specific CTL were not due to the biased usage of preceding Vβ segments, since this was also a characteristic of the HLA-B27 autoreactive CTL which, as mentioned above, showed very heterogenous HOP.

Thus, SHOP analysis of TCRB-CDR3 from HLA-B27-restricted autoreactive and Y. enterocolitica specific CTL revealed that much more CTL shared characteristic constraints within their CDR3 than it had been assumed by conventional sequence analysis. This further strengthens the hypothesis that both groups of CTL recognize limited sets of antigenic peptides. In addition, since identical HLA-restriction of both groups of CTL ruled out strictly MHC dictated conservations, we reasoned that the differences in TCRB-CDR3 SHOP profiles between these two functionally non-cross-reactive groups of CTL (29) might mirror distinct properties of the antigenic peptide(s). To test this hypothesis in the absence of known peptides recognized by the CTL studied,
we analyzed published HLA-B27 binding self peptides and HLA-B27 binding peptides from Y. enterocolitica. Supporting our assumption, we found that both groups of peptides were also characteristically dissimilar from each other. Since a previous report has outlined the importance of P6 of the peptide and P5 of the TCRβ-CDR3 for the interaction of the TCR with MHC class I presented ovalbumin (53), it may be of interest that the same positions showed a restricted distribution of hydropathy in the published HLA-B27 eluted self peptides and our HLA-B27-restricted autoreactive CTL. In addition, X-ray crystallography of HLA-B27 with bound self peptide also suggested that peptide P6 is directly accessible to the TCR (12). Discordant to the autoreactive CTL and self peptides, there was a clear preference for neutral and charged amino acids in TCRβ-CDR3 loops from Y. enterocolitica-specific CTL, particularly at P9–11. Concordantly, neutral and charged amino acids also predominated among Y. enterocolitica-derived HLA-B27 binding peptides (17). Although the exact nature and number of peptides recognized by our autoreactive CTL cannot be determined from this study, higher length variability in autoreactive and Yersinia-specific TCRβ-CDR3 compared to CDR3 with defined peptide specificity suggests that both groups of CTL recognize more than one peptide. Analysis of subgroups of autoreactive HLA-B27-restricted CTL demonstrated that eight out of 14 TCRβ-CDR3 sequences displayed an intriguingly concordant distribution of hydropathy in their hypervariable N-regions, suggesting that this subset was selected by one peptide or several peptides sharing similarly restricted hydropathy profiles. Epitope sharing should be taken into account particularly for the dual ionic state of histidine, and ignores space filling geometrical associations suggested by HOP analysis therefore need to be verified as soon as better methods are available. These limitations notwithstanding, HOP analysis of different groups of CTL with variable or defined peptide specificity selectively showed a high degree of homology in TCRβ-CDR3 of the latter CTL, supporting mounting experimental evidence (9,10,44) that the distribution of hydropathy in TCRβ-CDR3 may be crucially related to the capacity of the TCR to interact with the MHC bound peptide. Furthermore, limited and characteristically dissimilar HOP profiles of HLA-B27-restricted TCRβ-CDR3 from functionally non-cross-reactive autoreactive and Y. enterocolitica-specific CTL from ReA patients suggest that each group of CTL recognizes a smaller number of exclusive peptide antigens.

In summary, our data indicate that analysis of the distribution of hydropathy in TCRβ-CDR3 and groups of antigenic peptides can yield additional information that is likely to be biologically meaningful. Increasing evidence that physicochemical parameters of peptides and TCR rather than rigid sequence motifs determine T cell specificities will certainly affect research on T cell reactivity, induction of tolerance and molecular mimicry.

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Abbreviations

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<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tbody>
<tr>
<td>CTL</td>
<td>cytotoxic T lymphocyte</td>
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<tr>
<td>MP</td>
<td>matrix protein</td>
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<td>PBMC</td>
<td>peripheral blood mononuclear cells</td>
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<tr>
<td>ReA</td>
<td>reactive arthritis</td>
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<tr>
<td>RS</td>
<td>Reiter’s syndrome</td>
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<tr>
<td>SFMC</td>
<td>synovial fluid mononuclear cells</td>
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<tr>
<td>SHOP</td>
<td>superimposed hydropathy plots</td>
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References
Limited hydrophathy in HLA-B27-restricted TCRB-CD3


