Preferential Vβ Gene Usage and Lack of Junctional Sequence Conservation among Human T Cell Receptors Specific for a Tetanus Toxin-derived Peptide: Evidence for a Dominant Role of a Germline-encoded V Region in Antigen/Major Histocompatibility Complex Recognition

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Summary
To investigate the structural and genetic basis of the T cell response to defined peptide/major histocompatibility (MHC) class II complexes in humans, we established a large panel of T cell clones (61) from donors of different HLA-DR haplotypes and reactive with a tetanus toxin-derived peptide (tt830-844) recognized in association with most DR molecules (universal peptide). By using a bacterial enterotoxin-based proliferation assay and cDNA sequencing, we found preferential use of a particular VB region gene segment, VB2.1, in three of the individuals studied (64%, n = 58), irrespective of whether the peptide was presented by the DR6w2c, DR4w4, or DRw11.1 and DRw11.2 alleles, demonstrating that shared MHC class II antigens are not required for shared Vβ gene use by T cell receptors (TCRs) specific for this peptide. Vα gene use was more heterogeneous, with at least seven different Vα segments derived from five distinct families encoding α chains able to pair with VB2.1 chains to form a tt830-844/DR-specific binding site. Several cases were found of T cells restricted to different DR alleles that expressed identical Vβ and (or very closely related) Vα gene segments and that differed only in their junctional sequences. Thus, changes in the putative complementary determining region 3 (CDR3) of the TCR may, in certain cases, alter MHC specificity and maintain peptide reactivity. Finally, in contrast to what has been observed in other defined peptide/MHC systems, a striking heterogeneity was found in the junctional regions of both α and β chains, even for TCRs with identical Vα and/or Vβ gene segments and the same restriction. Among 14 anti-tt830-844 clones using the VB2.1 gene segment, 14 unique Vβ-D-Jβ junctions were found, with no evident conservation in length and/or amino acid composition. One interpretation for this apparent lack of coselection of specific junctional sequences in the context of a common V element, VB2.1, is that this V region plays a dominant role in the recognition of the tt830-844/DR complex.

Recognition by T cells of antigens presented in the form of short peptide fragments bound to self-MHC molecules is mediated through a clonotypic cell surface heterodimer receptor (TCR) composed of an α and β chain (1, 2). The TCR specificity resides in the amino terminal α and β variable domains which are generated as a result of combinatorial juxtaposition of germline-encoded V, D (for the β chain), and J gene segments and by somatic diversification mechanisms operating at the Vα-Jα and Vβ-D-Jβ junctions (reviewed in reference 3).

Amino acid sequence comparison of the α and β V regions with their Ig counterparts have shown a remarkable conservation of residues critical for maintaining the basic architecture of the Ig V regions (4, 5). Thus, although the three-dimensional structure of the TCR is not yet known, it has been predicted that its V regions fold and pair similarly to Ig V regions (3-5). Furthermore, the identification of hypervariable regions in both α and β subunits (more evident, however, for the β chain) at sites corresponding approximately to the Ig CDRs 1, 2, and 3 (6-8), suggests that in the TCR these regions contribute to the interaction with the peptide antigen/MHC complex. However, in contrast to IgS, somatic
mutations in the rearranged TCR V gene segments do not appear to play a significant role in the generation of diversity (9), implying, perhaps, that preservation of the germline-encoded CDRs is an important factor in maintaining TCR specificity.

Structural analysis of T cell responses to defined peptide/MHC class II complexes in the mouse has indicated that TCRs with the same specificity tend to use a limited set of V gene segments (10-18), and that the use of particular Vα (10, 11, 13) or Vβ (10-12) segments correlates with antigen or MHC recognition. Moreover, TCRs using the same V gene segments often have been expressed to very similar junctional sequences in both length and amino acid composition (14-18), arguing in favor of a key role of these junctional regions in the recognition of a given antigen/MHC class II complex.

In the present study we sought to obtain further insights into the molecular mechanism of antigen and MHC recognition. As a model system we chose to analyze the V region structural components of HLA-DR-restricted TCRs selected in response to an epitope defined by a short synthetic peptide comprising residues 830-844 of tetanus toxin (tt)1 (19). Previous investigations have established that this peptide is universally immunogenic, since it is recognized in all tetanus toxin (TT)-primed donors tested irrespective of their DR haplotype (20), and that it appears to bind to different DR molecules in a similar orientation as demonstrated by experiments using truncated and substituted peptide (20, 21); and by the fact that some (promiscuous) clones recognize the peptide bound to several (up to five) different MHC alleles (21, 22). This system therefore allows one to evaluate the impact of restricting element polymorphism on the structure of TCRs specific for the same immunogenic peptide. This polymorphism may determine both the way in which a peptide binds to a particular MHC molecule and its interaction with the TCR (23).

We established a large panel of anti-tt830-844 T cell clones restricted to the same or different DR alleles from several TT-primed donors with distinct HLA-DR haplotypes and characterized as previously described (20). The isotype of class II molecules recognized by each T cell clone was determined by antibody blocking experiments, and the DR restricting alleles were identified by using a panel of HLA-DR homozygous EBV B cells as APC (20).

Antigens. Peptide tt830-844, corresponding to amino acids 830-844 of tt (QVIKANSKFIGITEL), was mapped and synthesized as described (19). Native tt was obtained from Calbiochem Corp. (La Jolla, CA). TT was obtained from the Swiss Serum Institute (Bern, Switzerland). Purified Staphylococcus toxic shock system toxin 1 (TTST-1) was purchased from Toxin Technology Inc., (Madison, WI).

T cell Proliferation Assays. Cultures were set up in 200 μl RPMI-FCS in flat-bottomed microplates. T cells (3 x 10⁶) were cultured with 2 x 10⁴ irradiated (6,000 rad) EBV-transformed B cells. TT (1-100 μg/ml), peptide tt830-844 (0.01-20 μg/ml), or TSST-1 (0.1-50 ng/ml) were either added in the culture or used to pulse the EBV B cells. After 2 d at 37°C, 5% CO₂, the cultures were pulsed with 1 μCi [³H]thymidine (Amersham International, Bucks, UK) (sp act, 5 Ci/mmol), and incorporated radioactivity was measured after an additional 16 h by liquid scintillation.

PCR Amplification and Sequence Analysis of TCR-α and β cDNA. Total RNA was prepared from 5-10 x 10⁶ cells by the acid guanidinium thiocyanate-phenol-chloroform extraction method (24). Yields were usually 5-10 μg of total RNA for 10⁶ cells. A previously described anchored-PCR (A-PCR) technique (25) was used with slight modifications. Single-strand cDNA was obtained using reagents from a cDNA synthesis kit (Boehringer Mannheim Corp., Indianapolis, IN). 5 μg of total RNA was used for each synthesis that was performed in 20 μl for 1.5 h at 42°C according to the manufacturer’s instructions. The reaction mixture was then precipitated by isopropanol in 1 M ammonium acetate to eliminate free nucleotides and the oligo(dT) primer. A poly(dG) tail sequence was then added to the cDNA with terminal deoxynucleotidyl transferase (IBI, New Haven, CT) in a 40-μl reaction mixture containing a cobalt ³H buffer (IBI), and 0.1 mM dGTP for 30 min at 37°C.

Amplification of α and β variable regions were performed with Thermus aquaticus (Taq) polymerase (Perkin Elmer Cetus Corp., Norwalk, CT) by using 10% of the G-tailed cDNA in the standard buffer containing 50 pmol of each of two oligo-primers in a final volume of 100 μl. One of the two primers contained a sequence (underlined) complementary to the poly(G) (5'-CACCTGCAGCCGCCGCGTGACCCCCCCCCCCCCC), and the other contained a sequence complementary to either the 5’ end of α constant region (5'-GCCGAATTCAGAATTTAGCATGCGAAGCATGATGCTCAGTGGCTACTG), or the 5’ end of β constant region (5'-GCTCTAGAGATCGAGCCGCTTCGAGACTGATGTGGAGT-3'), and the latter primer and a
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jeered to a denaturation step at 94°C for 5 min, then to 25
AGGCGTCGAGAAGG-Y) were used to detect expression of V/82
V/82-specific primer (5'-GCGAATTCGTCGACATACGAGCA-
in GC clones. Amplification primers contained, in addition, re-
striction sites used for cloning. Each reaction mixture was sub-
were either the universal MD forward primer (U.S. Biochemical
Corp., Cleveland, OH) or ot or/8 specific primers: 5'-GCGAA-
T TCAGATCTTAGGCAGACAGACTTGTC ACTGC~Y, comple-
gluI site in the ot constant specific primer or in the/8 constant
region. Several independent MD subclones (usually 5-10) were se-
quences obtained from GenBank were performed by using the Fastp
sequences from donors AL and BK, respectively. For each allele, 10 and 20 independent sequences were determined
Since they are capable of efficiently recognizing
when cDNAs corresponding to the TCR/8 chains of a
donors expressing the same or different DR alleles (Table 1).
When cDNAs corresponding to the TCR/8 chains of a
number of clones from donors AL and BR were isolated by the A-PCR technique (25) and sequenced, it was found that the
majority used a V/82 gene family member, V/82.1. In both
donors we found two V/82.1 sequences, one that matched
previously published sequence (33), referred to as V/82.1a
(34), and the other that differed by a single nucleotide sub-
stitution resulting in an Arg instead of a Trp at amino acid
position 10 (numbering according to Kabat et al. [35], not
shown). This latter sequence is likely to represent an allelic
variant of V/82.1 that has been named V/82.1c, to distinguish it from a third allele, V/82.1b, that differs from V/82.1c by a
single amino acid (Lys42 substituted by Gln) (34).

Northern Blot Analysis of V/32 Expression. For each clone, 2.5
µg of total RNA was denatured at 65°C in formamide buffer, and
electrophoresed on 1.3% agarose gels containing 1.7% formalde-
hyde. RNAs were then transferred to nylon membranes (Hybond,
Amerham, UK), using a Fast tran apparatus (Genetex, Geneva,
Switzerland) and filters baked for 2 h at 80°C. Filters were pre-
hybridized for 2.5 h at 65°C in Church's buffer (0.5 M phosphate
buffer, pH 7.2, 7% SDS). Hybridization was performed for 4 h
at 65°C in the same buffer containing 2 × 106 cpm/ml of [32P]random-priming-labeled (31) V/82.1 cDNA probe. The probe
was derived from a human T cell clone (GM2.11) (20) that was
found to express V/82.1 as detected by nucleotide sequencing (data
not shown), and by specific PCR amplification as described above.
The amplified DNA was digested with PstI restriction enzyme to
remove the junctional region and V8 fragment purified on an agarose
gel. Hybridized filters were washed twice in 2 × SSC, 0.1% SDS,
then once in 1 × SSC, 0.1% SDS at 65°C. Membranes were ex-
posed overnight to Kodak X-OMAT AR film using an intensifying
screen.

HLA-DR Typing. The DR phenotyping of donors GC, KK,
GAR, and BDM was assigned by serological typing (20). The DR
phenotype of GC was also determined by PCR and hybridization
with a DRw11.1-specific oligonucleotide (Dr. G. Mazzola, Torino,
Italy, personal communication). To obtain the precise HLA-DR
type expressed by donors AL (previously reported to be homozy-
gous DRw1l [w6] by serological typing [20]) and donor BR, typing
was performed by PCR amplification and sequencing of the first
domain of the HLA-DR/8 chain using cDNA prepared as described
above. Amplification primers were 5'-GTTCCTAGAGTCGACG-
GGACCCGGCCACGCAGTTCC-3' and 5'-CAGAATTCTGG-
ATCCTCAGGCCCTGCACGTGGAAC-3', complementary to the
5' and 3' regions of the first domain of the HLA-DR/8 chain, respec-
tively, (32) which allowed amplification of HLA-DR/8 (α1, β, and
β4) isotypes. Thus, to obtain at least a few representative sequences
for each allele, 10 and 20 independent sequences were determined
donors AL and BR, respectively.

Results

Preferential Use of a V/32 Family Member among DR-restricted
TCR Clones Specific for tt830-844. 61 anti-tt830-844 DR-
restricted T cell clones were isolated from six TT-primed donors expressing the same or different DR alleles (Table 1).
Some of these clones express a promiscuous pattern of recognition
(20), since they are capable of efficiently recognizing
the autologous DR, as well as by other DRs. Thus, for example, certain clones from donor AL (homozygous DRw6c1) react with peptide presented by the DRw11.2 molecule in addition to the autologous DR.

When cDNAs corresponding to the TCR/β chains of a
number of clones from donors AL and BR were isolated by the A-PCR technique (25) and sequenced, it was found that the
majority used a V/82 gene family member, V/82.1. In both
donors we found two V/82.1 sequences, one that matched
previously published sequence (33), referred to as V/82.1a
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stitution resulting in an Arg instead of a Trp at amino acid
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single amino acid (Lys42 substituted by Gln) (34).

T cells expressing V/82 have been reported to proliferate in response to TSST-1 superantigen bound to class II MHC molecules (36). To determine whether this property could be used to rapidly and reliably check for V/82 expression, 16
AL clones were tested both for their ability to respond to TSST-1 presented by autologous EBV-transformed B cell lines, and for V/82.1 expression by Northern blot hybridization analysis.

The results presented in Fig. 1 demonstrate a perfect correlation between a proliferative response to TSST-1 and expression of V/82.1. These data also indicate that the substitution at amino acid position 10 that accounts for the two
V/82.1 alleles does not affect the interaction with TSST-1. Altogether, 13 of 21 clones (~60%) from AL and four of eight (50%) from BR all restricted to DRw6c1, proliferated in response to TSST-1 (Table 1). In addition, four of eight clones (50%) from donor BR that recognized tt830-844 in the context of DR4w4 were positive in the TSST-1 stimulation
assay (Table 1).

Sequencing of TCR-β cDNAs of 15 TSST-1-responsive clones from donors AL and BR indicated that, as expected, all expressed V/82.1 (Table 2). In contrast, 14 unique V/β-D-
β junctional sequences were found (Fig. 2). Clones AL8.1 and AL12.1, which used the same Vα and Vβ gene segments and had identical α and β junctional sequences, were derived

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Table 1. Frequency of Vβ2.1-positive Anti-tt (830-844) T Cell Clones Isolated from Different Donors

<table>
<thead>
<tr>
<th>Donor (HLA-DR haplotype)*</th>
<th>HLA-DR* restriction of T cell clones</th>
<th>Frequency of Vβ2.1* T cell clones†</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>AL</td>
<td>DRw6c1, DRw11.2</td>
<td>7/8</td>
<td></td>
</tr>
<tr>
<td>(DRw6c1, DRw6c1)</td>
<td>DRw6c1</td>
<td>6/13</td>
<td>13/21</td>
</tr>
<tr>
<td>BR</td>
<td>DRw6c1, DRw11.1, DRw11.2</td>
<td>2/2</td>
<td></td>
</tr>
<tr>
<td>(DRw6c1, DRw4w4)</td>
<td>DRw6c1, DRw11.1</td>
<td>1/1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>DRw6c1, DRw11.2</td>
<td>0/1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>DRw6c1</td>
<td>1/4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>DRw4w4</td>
<td>4/8</td>
<td></td>
</tr>
<tr>
<td>GC</td>
<td>DRw11.1</td>
<td></td>
<td>16/21</td>
</tr>
<tr>
<td>(DRw11.1, DRw11.1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KK</td>
<td>DR11.1</td>
<td></td>
<td>0/1</td>
</tr>
<tr>
<td>(DR11.1, DR3)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GAR</td>
<td>DRw11.1</td>
<td></td>
<td>0/1</td>
</tr>
<tr>
<td>(DRw11.1, DR1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BDM</td>
<td>DRw11.1</td>
<td></td>
<td>0/1</td>
</tr>
<tr>
<td>(DR11.1, DRw11.1)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Most of the clones proliferated in response to low concentration of peptide (0.02-1 μg/ml) as well as to the native tt molecule presented by autologous APC, demonstrating that these clones are indeed tetanus specific.

*The nomenclature of each DR allele is according to reference 32.
†Vβ2 expression was assessed by TSST-1 proliferation assay (described in Materials and Methods) for all the clones indicated except for three clones from donor GC where Vβ2.1 expression was determined by PCR using Vβ2.1- and Cβ-specific primers (data not shown).

from two independent in vitro primary cultures, strongly suggesting that the same clone present in PBMC had been independently isolated twice. Thus, 14 anti-tt830-844 clones from two donors, each expressing an independent β rearranging event, used Vβ2.1.

To determine whether other Vβ germline gene segments were preferentially used in the anti-tt830-844 response, a sample of eight TSST-1-unresponsive clones (three each from donors AL and BR, and one each from donors KK and GAR; Table 1) was subjected to sequence analysis. As expected, Vβ2.1 was not expressed in any of these clones (Table 2). Furthermore, in contrast to the repeated usage of Vβ2.1, no selected use of other Vβ gene segments emerged since the AL clones expressed Vβ21.3, Vβ6.9, and Vβ19.1, the BR clones Vβ8.3, Vβ13.6 (a new member of this gene family; Fig. 3), and Vβ7.2, and the KK and GAR clones Vβ12.3 and Vβ19.1, respectively. No significantly greater degree of amino acid sequence identity could be demonstrated between Vβ2.1 and any of these gene segments (~30%), than between Vβ2.1 and members of other Vβ families.

As Vβ2.1 was found to be used by TCRs restricted to two distinct DR alleles (DRw6c1 and DR4w4), we tested whether anti-tt830-844 clones isolated from a third donor, GC (homozygous DRw11.1), also preferentially used Vβ2.1. This haplotype was particularly interesting as 10 of 17 Vβ2.1-positive, DRw6c1-restricted clones from individuals AL and BR were promiscuous for DRw11.1 and/or DRw11.2 (Tables 1 and 2). It was found that 16 of 21 clones (76%) from donor GC expressed Vβ2.1, as indicated by proliferation to TSST-1 and/or PCR (Table 1).

In conclusion, the above results indicate that, at least in certain individuals, Vβ2.1 is strongly selected for recognition of tt830-844 whether presented by any of three different DR alleles, and suggest that this V segment may be interacting with a structural determinant common to these DR complexes.

Anti-tt830-844 Clones Display a Profile of Vα Gene Use that May Be Particular to Each Donor. We then investigated whether, as observed for Vβs, particular Vα family members were also selected in response to tt830-844 by determining the nucleotide sequences of the Vα segments expressed in clones whose Vβs had been characterized (Table 2). Two distinct α sequences were often found in a given clone, although in two-thirds of the cases one of the two was the result of a nonproductive rearrangement (i.e., Vα-Jα junction not in frame, indicated by † in Table 2). In clones AL4.1, ALIII6.3, BR7.5, and GAR9.2, two functional α chains were detected. In each of these clones, sequencing of 8-10 independent β cDNAs did not reveal the presence of a second Vα suggesting that the expression of two α chains is likely to be due to
lack of allelic exclusion at the level of \(\alpha\) rearrangement (28), rather than to contamination by a second T cell clone. In the remaining clones, a single \(\alpha\) could be detected in 5–10 independent cDNAs sequenced. It is of interest that, besides the unique human \(\alpha_\alpha\) sequence described (43), we found a variant in individual AL in which \(\alpha\) at position 120 of the C region is replaced by Lys (Fig. 2) as the result of a single nucleotide substitution. This could correspond to an allelic variant of \(\alpha\), because in the AL donor, both forms were found in different clones and, when two \(\alpha\) sequences (out of frame, not shown, or in frame, Fig. 2) were present in a given clone, each of them expressed one of these two forms. This further reinforces the notion of lack of allelic exclusion for \(\alpha\) chain rearrangement.

As shown in Table 2, clones from donor AL preferentially used the \(\alpha_21.1\) and \(\alpha_{17.1}\) family members, each in at least 3 and potentially 4 of 10 clones, whereas in the BR donor, two members of the \(\alpha_2\) family, \(\alpha_{2.2}\), and \(\alpha_{2.6}\), are expressed in 4 (or 5) of 10 clones. Thus, individuals AL and BR display similar high frequencies of \(\beta_2.1\) use (≥50%), and their profile of \(\alpha\) use is more heterogeneous and shows considerably less overlap. Some shared use of \(\alpha\) gene segments, however, does occur, in that \(\alpha_1\) family members are expressed in clones AL9.2, BR7.3, and BR22.5, and \(\alpha_8\) family members are found in clones AL8.1, BR1.7, BR7.5, and GAR9.2. Nevertheless, certain gene segments appear to be preferentially used in each individual. A new \(\alpha\) family (\(\alpha_{30}\), a new member of the \(\alpha_{14}\) family (\(\alpha_{14.1}\)), and a new J(\(\alpha\)) gene segment (AL 6.3) were identified (Fig. 3).

Multiple Combinations of \(\alpha\) and \(\beta\) Gene Segments Are Found to Constitute TCRs Specific for \(\text{tt}330-344\). As shown in Table 2, a rather diversified repertoire of \(\alpha\) germline gene segments coexpressed with \(\beta_2.1\) was evident, although repeated use of the same or a related \(\alpha\) was found in a number of cases. Thus, in donor AL, \(\alpha_21.1\) was expressed in clones AL15.1 and AL11.6.1, and \(\alpha_{17.1}\) was found in clones AL11.4.3, AL17.1, and AL4.1, although in the latter clone \(\alpha_{14.2}\) could also potentially pair with \(\beta_2.1\). In donor BR, two \(\alpha_2\) members, \(\alpha_{2.2}\) and \(\alpha_{2.6}\), which are 72% identical at the amino acid level, were found associated with \(\beta_2.1\) in two and, potentially three, DR6w6c-restricted clones. In both donors, two additional \(\alpha\) gene families, \(\alpha_8\) and \(\alpha_1\), could also pair with \(\beta_2.1\). Altogether, at least seven different \(\alpha\) gene segments derived from five distinct families can encode \(\alpha\) chains able to pair with \(\beta_2.1\) chains to form a \(\text{tt}330-344/\text{DR}\)-specific binding site. Of note, however, is that \(\alpha_{21.1}\), \(\alpha_{17.1}\), \(\alpha_{2.2}\), and \(\alpha_{8.1}\) (\(\alpha_{8.2}\)), among the \(\alpha\) segments found in association with \(\beta_2.1\), display a
## Table 2. V and J Gene Segments Used by the α and β Chains of Anti-tt830-844 TCRs in Clones from Donors AL, BR, and KK, GAR

<table>
<thead>
<tr>
<th>Clone T</th>
<th>HLA-DR Restriction</th>
<th>Vα</th>
<th>Jα</th>
<th>Vβ</th>
<th>Jβ</th>
</tr>
</thead>
<tbody>
<tr>
<td>AL 15.3</td>
<td>DRw6cI P1</td>
<td>Va21.1</td>
<td>JaR1</td>
<td>Vβ2.1a</td>
<td>Jβ1.2</td>
</tr>
<tr>
<td>AL III6.1</td>
<td>DRw6cI</td>
<td>Va21.1</td>
<td>JaU7</td>
<td>Vβ2.1c</td>
<td>Jβ1.1</td>
</tr>
<tr>
<td>AL III4.3</td>
<td>DRw6cI</td>
<td>Va17.1</td>
<td>JaAF211</td>
<td>Vβ2.1</td>
<td>Jβ2.1</td>
</tr>
<tr>
<td>AL 17.3</td>
<td>DRw6cI</td>
<td>Va17.1</td>
<td>JaF4</td>
<td>Vβ2.1c</td>
<td>Jβ2.7</td>
</tr>
<tr>
<td>AL 4.1</td>
<td>DRw6cI P1</td>
<td>Va17.1</td>
<td>JaAC9</td>
<td>Vβ2.1a</td>
<td>Jβ1.5</td>
</tr>
<tr>
<td>AL 8.1</td>
<td>DRw6cI P1</td>
<td>Va8.1</td>
<td>JaIGRJa06</td>
<td>Vβ2.1c</td>
<td>Jβ2.7</td>
</tr>
<tr>
<td>AL 12.1</td>
<td>DRw6cI</td>
<td>Va1.10</td>
<td>JaAA17</td>
<td>Vβ2.1c</td>
<td>Jβ1.2</td>
</tr>
<tr>
<td>AL 9.2</td>
<td>DRw6cI</td>
<td>Va1.10</td>
<td>JaIGRJa10</td>
<td>Vβ2.1c</td>
<td>Jβ2.5</td>
</tr>
<tr>
<td>AL III3.1</td>
<td>DRw6cI</td>
<td>Va21.1</td>
<td>JaAF211</td>
<td>Vβ2.1a</td>
<td>Jβ2.5</td>
</tr>
<tr>
<td>AL III6.3</td>
<td>DRw6cI</td>
<td>Va17.1</td>
<td>JaAC17</td>
<td>Vβ6.9</td>
<td>Jβ2.5</td>
</tr>
<tr>
<td>AL 7.1</td>
<td>DRw6cI P1</td>
<td>Va17.1</td>
<td>JaG</td>
<td>Vβ19.1</td>
<td>Jβ2.7</td>
</tr>
<tr>
<td>BR 2.2</td>
<td>DRw6cI P2</td>
<td>Va8.2</td>
<td>JD</td>
<td>Vβ2.1c</td>
<td>Jβ1.2</td>
</tr>
<tr>
<td>BR 7.5</td>
<td>DRw6cI P3</td>
<td>Va8.2</td>
<td>JaK</td>
<td>Vβ2.1a</td>
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<td>BR 9.13</td>
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<td>Va2.6</td>
<td>JaR2</td>
<td>Vβ2.1c</td>
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</tr>
<tr>
<td>BR 15.3</td>
<td>DRw6cI</td>
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<td>Va1.2</td>
<td>JaAC17</td>
<td>Vβ2.1a</td>
<td>Jβ2.5</td>
</tr>
<tr>
<td>BR 22.5</td>
<td>DRw4w</td>
<td>Va1.2</td>
<td>JaU</td>
<td>Vβ2.1c</td>
<td>Jβ2.4</td>
</tr>
<tr>
<td>BR 1.7</td>
<td>DRw4w</td>
<td>Va8.1</td>
<td>JaS</td>
<td>Vβ2.1c</td>
<td>Jβ2.3</td>
</tr>
<tr>
<td>BR 5.11</td>
<td>DRw4w</td>
<td>Va8.1</td>
<td>JaGRJa06</td>
<td>Vβ13.6</td>
<td>Jβ2.3</td>
</tr>
<tr>
<td>BR 1.3</td>
<td>DRw4w</td>
<td>Va6.1</td>
<td>JaG</td>
<td>Vβ19.1</td>
<td>Jβ2.2</td>
</tr>
<tr>
<td>KT 2</td>
<td>DRw11.1</td>
<td>Va30.15</td>
<td>JaIGRJa13</td>
<td>Vβ12.3</td>
<td>Jβ2.2</td>
</tr>
<tr>
<td>GAR 9.2</td>
<td>DRw11.1</td>
<td>Va8.2</td>
<td>JaS</td>
<td>Vβ19.1</td>
<td>Jβ1.2</td>
</tr>
</tbody>
</table>

P Indicates promiscuous clones which, in addition to autologous DR recognize ttt830–844 presented in the context of DRw11.2 (P1), DRw11.1, and DRw11.2 (P2), or DRw11.1 (P3).

* All the following V gene segments found in anti-tt830–844 clones have been reported by Wilson et al. (38); the original sequence designation is indicated in parentheses: Vα1.2 (PY4), Vα2.2 (AP110), Vα2.6 (AABS), Vα6.1 (HAFP01), Vα8.1 (HAPB15), Vβ2.1 (HAPB50), Vα17.1 (AB111), Vα8.2 (AB111), Vα8.1 (AB111), Vα21.1 (AF211), Vβ2.1 (PL2.13), Vβ6.9 (L17), Vβ7.2 (PL4.19), Vβ8.3 (VBH33), Vβ12.3 (PH27), Vβ17.1 (PH29), and Vβ19 (HBVT06). Vβ2.1 was described in reference (39). The Jβ segments were described by Toyonaga et al. (40). Jα sequences were assigned to previously described Jα gene segments (37, 41, 42).

Clones in which a second, out-of-frame, sequence was detected.

Sequences corresponding to new V and J segments not yet described: Vα14.2, Vα30.15, Jα8.1, and Vβ13.6 (see Fig. 3).

1 A second in-frame Vβ sequence (Vβ17.1-Jβ2.6) has been found in one of nine independent sequences from this clone.

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... significantly higher degree of similarity to each other (50–60% amino acid identity) than to members of other Vα families (<40%), with the exception of Vα15 and Vα23 (not shown).

An interesting situation was noticed for the three Vβ2.1-positive clones from BR restricted to DR4w4. Clone BR1.7 shares the same Vα gene segment, Vα8.1, as the DRw6cI-restricted clone AL8.1. Similarly, it was found that clones BR7.3 and 22.5 expressed Vα1.2, which is closely related to the Vα1.10 segment (89% homologous at the amino acid level) used by the DRw6cI-restricted clone AL9.2. However, the TCRs of these pairs of clones differ significantly at their corresponding junctional regions (Fig. 2). These observations suggest that germline-encoded structural components of the TCR may not be responsible for recognition of the DR polymorphic residues, at least in this particular case, and that junctional region residues can influence MHC specificity.

The complexity of Vα and Vβ combinations selected for recognition of the peptide/DR complexes studied is further...
Figure 2. Amino acid sequence alignment of α and β V-(D)-J regions. For each clone are indicated the names of the corresponding Vα, Jα, Vβ, and Jβ segments. Only the last three amino acid residues of each V segment are shown, followed by the junctional sequences and the first three or four residues of the constant region. Amino acid residues are indicated using a single-letter code. The assignment of the CDR3 loop is according to Chothia et al. (5). J sequences contributing to each CDR3 are underlined.
Figure 3. Nucleotide sequences and corresponding amino acid translation of new human variable and joining segments. (A) A proposed new Vα family, Vα30, expressed in clone KT2. When compared to all known human Vα sequences, Vα30 shows the highest degree of nucleotide sequence identity (69%) with one member of the Vα10 family (38). (B) Vα4.2 (from clone AL4.1) and (C) Vβ13.6 (from clone BR5.11) correspond to new sequences belonging to already described families. Vα4.2 shares 93.5% nucleotide identity with the HAVT20 prototype (38) of the Vα10 family. Vβ13.6 is a partial sequence but shows 90-96% nucleotide identity with the previously described sequences of the Vβ313 family HBP34 (38), CEM (38), 17A2 and G36 (44). The presumed leader sequence of Vα30 and Vβ14.2 is overlined. These new sequences have been submitted to the GenBank nucleotide data base and have been assigned the accession numbers: M64350 (Vα30), M64354 (Vβ14.2), and M64355 (Vβ13.6). (D) A new Jα segment, designated JαAL-6.3, according to the name of the corresponding clone (ALIII6.3).
In the Vβ2.1 chain of clone AL8.1, a conservative Lys for Arg substitution has taken place. However, it is evident that the putative Vβ CDR3s of the anti-tt830-844 TCRs analyzed show dramatic differences in both length (6–16 residues) and/or amino acid composition. This is observed even for the junctional regions of TCRs that use members of the same Vα family and the same Vβ, and that are restricted to the same DR, such as clones ALIII4.3, AL17.3, and AL4.1, or BR15.3, BR9.13, and BR7.5 (for AL 4.1 and BR7.5, it is tentatively assumed that Vα17.1 and Vα2.6, respectively, each pair with Vβ2.1), or clones AL15.3 and ALIII6.1. The only exception to this striking lack of junctional sequence conservation is represented by clones BR7.3 and BR22.5, which are both restricted to DR4w4, where the use of the same Vα and Vβ gene segments is accompanied by relatively conserved Vα CDR3s, both in amino acid composition and in length. Their Vβ CDR3s, however, differ markedly.

In summary, among the 23 independent clones analyzed, 22 unique Vβ-D-Jβ and 25 unique Vα-Jα junctions were found and, with a single exception, no greater junctional sequence conservation was observed among TCRs with identical Vα and/or Vβ gene segments than among those with different ones.

Discussion

Previous studies in the mouse system (10–18) have demonstrated that, with a notable exception (45), TCRs selected in response to a defined peptide-Ia complex use a restricted number of Vα/Vβ gene segment combinations. In the one exception, involving the response to a determinant from influenza virus hemagglutinin, a markedly more diverse TCR repertoire was found. In all these studies, however, TCRs that used the same V region germline gene segments generally expressed closely related (or identical) junctional region amino acid sequences, suggesting that V regions and junctional sequences are functionally coselected. Thus, in the cytochrome c system (15), among nine murine clones expressing Vβ3, all were found to have the same number of amino acids spanning the Vβ-D-Jβ junction, and eight had an asparagine at position 100. Indeed, site-directed mutagenesis of this residue was shown to affect recognition (46).

To investigate whether a similar structural conservation of TCRs specific for a defined antigen/MHC complex is found in antigen-primed human donors and, at the same time, to obtain further insights into the molecular mechanism of antigen and MHC recognition, we analyzed the TCRs selected in a DR-restricted memory T cell response to the tt epitope tt830-844. Since tt830-844 can be recognized in association with most DR molecules (20), this system also offered an opportunity to evaluate the influence of changing the antigen-presenting molecule on the selection of TCR variable region components. In addition, the tt830-844 peptide seems to bind to different DR molecules in a similar orientation (20–22). This should simplify interpretation of the observed response. Our analysis of a large panel of anti-tt830-844 human T cell clones has revealed repeated use of a particular Vβ region gene segment, as often observed in the mouse system. In contrast to previous studies, however, no apparent conservation in the length and/or amino acid composition of Vα or Vβ junctional regions could be detected, even among TCRs with identical Vα and/or Vβ gene segments.

A particular Vβ gene product, Vβ2.1, was preferentially used by anti-tt830-844-specific TCRs, irrespective of whether the peptide was presented by DR6wcl, DR4w4, or DR11.1 and DR11.2 alleles in three of the donors studied. Thus, shared MHC class II antigens are not required for shared Vβ gene usage by TCRs specific for this peptide. This result is consistent with the finding by Wucherpfennig et al. (47) that TCRs reactive with an MBP-derived peptide and expressing Vβ12 or Vβ17 can be isolated from DR2-, DR3-, or DR4-positive patients. As in the present study, preferential use of a particular Vβ gene segment (Vβ17) in T cell clones specific for this peptide was also found. Another recently described case of restricted TCR gene expression in humans is that of Vα17 use by tumor-infiltrating lymphocytes in uveal melanoma (48). In this case, however, the nature of the targeted antigen(s) is unknown. In the anti-tt830-844 response, no preferential use of other Vβs was found in a series of clones not expressing Vβ2.1, suggesting that the latter is strongly selected, at least in the individuals studied, for interacting with a structural determinant common to the different peptide/DR complexes recognized. These complexes share an identical DR α chain, but the corresponding DR β chain display 3–11 amino acid substitutions when com-
pared with one another (Fig. 4). According to the proposed structural model of class II MHC (23), some of these polymorphic residues (on the floor of, or pointing inside the MHC pocket) may influence the way in which the peptide binds to the restricting element, and others (on the top of the DRβ α helix) may interact with the TCR and are unlikely to influence the determinant seen by Vβ2.1. Moreover, t.t830–844 has been shown to interact with different DR alleles via common residues (21). It is therefore likely that the promiscuous binding of this peptide is the result of the interaction between these residues and sites conserved in different DR molecules (20, 21). Given this apparently similar orientation of the peptide in the DR molecules, one would expect that different complexes share, at least in part, a common T cell determinant that can be seen by Vβ2.1. This hypothesis is favored by the observation that, with one exception, all clones displaying promiscuous recognition use Vβ2.1 (see Table 2). However, further studies will be needed to clarify whether the determinant selecting Vβ2.1 is borne by the nonpolymorphic α chain of DR, or by a common motif of the DR β chain, or rather of the peptide, or of both the peptide and DR molecule.

The Vα use in the 21 clones analyzed was not biased to only one Vα chain, as observed for Vβ. For instance, if we consider the 10 clones using Vβ2.1 and restricted to DRw661, a minimum of six (to a maximum of eight) different Vα family members could be used to recognize this complex. It is worth noting, however, that Vα2.2, Vα8.1 (Vα8.2), Vα17.1, and Vα21.1, which are among the Vα gene segments most frequently associated with Vβ2.1, display a significantly higher degree of similarity to each other (50–60% amino acid identity) than to most Vα members (<40% identity). Thus, one may speculate that they all share a minimal structural element important in forming a similar antigenic specificity and/or restriction. This possibility is consistent with what is known from sequencing and x-ray crystallographic analysis of anti-phosphorylcholine (49) and anti-phényloxyazolone (50) antibodies about how a particular binding specificity may be preserved, even after substitution of a Gln by a Pro, which may significantly affect the conformation of the CDR3 loop (53). Moreover, the Vβ CDR3s of these clones differ markedly.

Recently, two studies in the mouse investigating class I-restricted responses to a lymphocytic choriomeningitis virus (LCMV) undecapeptide in a transgenic system (54), and to a Plasmodium berghei nonapeptide (55), found substantially different Vβ-D-Jβ junctional regions in the context of a common Vβ. Thus, together with ours, these results indicate that variability in the junctional regions of TCRs specific for an identical peptide/MHC complex is not exceptional and may depend on the particular peptide (or epitope) recognized.

One interpretation for the apparent lack of selection of specific junctional sequences in the context of a common V (Vβ and/or Vα) region element is that the junctional regions of these TCRs do not play a major role in recognition of the t.t830–844/DR complex and so are not structurally constrained. That the putative CDR3 regions have at least some influence on recognition, however, is shown by the effect of
changes in these regions on DR restriction, as discussed above. In addition, changes in the junctional sequences may alter the affinity or fine specificity of these TCRs. Nevertheless, the strong selection for Vβ2.1, but not for any particular junctional sequence(s), found in many anti-tt830-844 clones suggests that the former may be responsible for most of the key contacts with the peptide/MHC complex. It is instructive to examine this situation in the light of what is known about the selection of particular CDR sequences in antibodies. In this case, X-ray crystallographic analysis has shown that the strict maintenance of certain amino acid sequences, such as those at the potentially highly variable Vc-Jc and Vc-D-Jc junctions (CDR3s) observed among anti-phosphorylcholine junctions (CDR3s) observed among anti-phosphorylcholine changes in these regions on DR restriction, as discussed above. Furthermore, these clones are all affected by substitutions in the central region of the tt830-844, indicating that they interact with the same stretch of the peptide. Finally, it is conceivable that in our particular case the peptide/MHC complex may be heavily tilted toward the Vβ domain and yet maintain the same overall alignment relative to the latter, as in the proposed models (4, 5, 56). This would have the effect of partially disengaging Vβ CDR3 (as well as the entire Vα domain) from the interface between the TCR and the peptide-MHC complex, and potentially allowing for greater sequence variability at the Vβ-D-Jβ junction. It is interesting that cases of asymmetrical positioning of the antibody heterodimer on the surface of antigen, resulting in markedly unequal contributions of Vc versus Vn domains to formation of the interface with antigen, have been well documented in X-ray crystallographic studies of antigen-antibody complexes (57, 58).

Further experiments involving the use of analogues of tt830-844 carrying a large number of substitutions, as well as TCR reconstitutions by transfection employing different combinations of α and β chains, should help to distinguish among these possibilities.

To our knowledge, the present study represents the most extensive analysis to date illustrating the complexity of TCR structural components repertoire selected for a well-defined epitope in the human. In addition, this system represents a useful model for further understanding the rules governing T cell recognition.

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