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 Vβ region gene segment, Vβ2.1, in three of the individuals studied (64%, $n = 58$), irrespective of whether the peptide was presented by the DR6wcI, 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 Vβ2.1 chains to form a tt830–844/DR-specific binding site. Several cases were found of clones 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 Vβ2.1 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, Vβ2.1, 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

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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 found to express 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 toxoid (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 asked whether and at which level structural constraints are imposed on the corresponding TCRs. Our results indicate that a particular Vβ region, encoded by the Vβ2.1 gene segment, is strongly selected in different individuals in the anti-tt830–844 response regardless of the DR allele (DRw6cI, DR4w4, DRw11.1, and DRw11.2) presenting the antigen. This suggests that this Vβ region may interact with a common structural element of these complexes. We then analyzed the Vα segments expressed by 14 Vβ2.1-positive clones, most of them restricted to the DRw6cI allele, and found that at least seven different ones could be used to form anti-tt830–844 specificities. It is surprising to note that, in contrast to the apparent coselection of similar junctional sequences in the context of common V elements reported in other model systems, the junctional regions of both the α and β chains of these TCRs were extremely diversified, even for TCRs using the same Vα and Vβ gene segments and restricted to the same DR molecule. These data indicate a limited conservation of TCR V region components selected in response to the antigen/MHC complexes studied, and are discussed in the context of possible models of TCR/Ag/MHC recognition.

[1] *Abbreviations used in this paper:* A-PCR, anchored polymerase chain reaction; MBP, myelin basic protein; TSST-1, Staphylococcus toxic shock syndrome toxin 1; tt, tetanus toxin; TT, tetanus toxoid.

Materials and Methods

Isolation of Peptide-specific T Cell Clones. The tt830–844-specific T cell clones were isolated from PBMC of HLA-typed TT-primed donors, 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 (QYIKANSKFIGITEL), 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) (TSST-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 (3×10^4) were cultured with 2×10^4 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% CO_2, the cultures were pulsed with 1 μCi [^{3}H]thymidine (Amersham International, Amersham, 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 $5–10 \times 10^6$ cells by the acid guanidinium thiocyanate-phenol-chloroform extraction method (24). Yields were usually 5–10 μg of total RNA for 10^6 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 sscDNA with terminal deoxynucleotidyl transferase (IBI, New Haven, CT) in a 40-μl reaction mixture containing a cobalt TdT 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 sscDNA 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′-CACTCGAGCGGCCGCGTCGACCCCCCCCCC-3′), and the other contained a sequence complementary to either the 5′ end of α constant region (5′-GCGAATTCAGATCTTAGGCAGACAGACTTGTCACTGG-3′), or the 5′ end of β constant region (5′-GCTCTAGAGTCGACGGCTGCTCAGGCAGTATCTGGAGT-3′). The latter primer and a

Vβ2-specific primer (5'-GCGAATTCGTCGACATACGAGCA-AGGCGTCGAGAAGG-3') were used to detect expression of Vβ2 in GC clones. Amplification primers contained, in addition, restriction sites used for cloning. Each reaction mixture was subjected to a denaturation step at 94°C for 5 min, then to 25 amplification cycles in a thermocycler (Hybaid, Teddington, Middlesex, UK), each consisting of a 10-s denaturation step at 94°C, a 1-min annealing step at 55°C, a 15-s primer stabilization at 60°C, and a 45-s elongation step at 72°C. A final extension step at 72°C for 5 min terminated the reaction.

PCR products were digested with SalI and BglII restriction enzymes. The SalI site was located in the anchor-oligo-primer and BglII site in the α constant specific primer or in the β constant region. Amplified products (of ~600–700 bp) containing α or β variable regions were agarose gel purified, subcloned into the M13mp19 bacteriophage vector, and sequenced according to the dideoxynucleotide termination method (26). Sequencing primers were either the universal M13 forward primer (U.S. Biochemical Corp., Cleveland, OH) or α or β specific primers: 5'-GCGAA-TTCAGATCTTAGGCAGACAGACTTGTCACTGG-3', complementary to the α constant region; 5'-TCTGCTTCTGATGGCT-CAAAC-3', complementary to the β constant region; 5'-CCG-GGGTATTGCACATACCA-3', 5'-CCAGGATCCTGTCTGTACCA-3', and 5'-CAAGGGTCCTGCCGAAACCA-3', degenerated primers complementary to a consensus sequence located in the α variable region and 5'-CCCAGGGTCTGTCGGTACCA-3', a degenerated primer complementary to a consensus sequence in the β variable region. Several independent M13 subclones (usually 5–10) were sequenced because PCR amplification is known to introduce errors through base misincorporation (27), and because the α (and possibly the β) loci of both chromosomes may undergo functional rearrangements (28, 29). Sequence homology comparisons with sequences obtained from GenBank were performed by using the Fastp program (30), and MV10000 computer facilities (Centre de calcul, Pasteur Institute, Paris, France).

Northern Blot Analysis of Vβ2 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% formaldehyde. RNAs were then transferred to nylon membranes (Hybond, Amersham, UK), using a Fast trans apparatus (Genofit, Geneva, Switzerland), and filters baked for 2 h at 80°C. Filters were prehybridized 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 2×10^6 cpm/ml of [^{32}P]random-priming-labeled (31) Vβ2.1 cDNA probe. The probe was derived from a human T cell clone (GM2.11) (20) that was found to express Vβ2.1 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 Vβ 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 exposed 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 allele expressed by donors AL (previously reported to be homozygous DRw13 [w6] by serological typing [20]) and donor BR, typing was performed by PCR amplification and sequencing of the first domain of the HLA-DR β chain using cDNA prepared as described above. Amplification primers were 5'-GTTCTAGAGTCGACG-GGGACACCGGACCACGTTCC-3' and 5'-CACGAATTCGG-ATCCTCGCCGCTGCACTGTGAAGC-3', complementary to the 5' and 3' regions of the first domain of the HLA-DR β chain, respectively, (32) which allowed amplification of HLA-DR β1, β3, and β4 isotypes. Thus, to obtain at least a few representative sequences for each allele, 10 and 20 independent sequences were determined from donors AL and BR, respectively.

Results

Preferential Use of a Vβ2 Family Member among DR-restricted T Cell 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 tt830–844 presented by the autologous DR, as well as by other DRs. Thus, for example, certain clones from donor AL (homozygous DRw6cI) 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 Vβ2 gene family member, Vβ2.1. In both donors we found two Vβ2.1 sequences, one that matched a previously published sequence (33), referred to as Vβ2.1a (34), and the other that differed by a single nucleotide substitution 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 Vβ2.1 that has been named Vβ2.1c, to distinguish it from a third allele, Vβ2.1b, that differs from Vβ2.1c by a single amino acid (Lys^{42} substituted by Gln) (34).

T cells expressing Vβ2 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 Vβ2 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 Vβ2.1 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 Vβ2.1. These data also indicate that the substitution at amino acid position 10 that accounts for the two Vβ2.1 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 DRw6cI, 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 Vβ2.1 (Table 2). In contrast, 14 unique Vβ-D-Jβ 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

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

Donor (HLA-DR haplotype)*	HLA-DR* restriction of T cell clones	Frequency of Vβ2.1⁺ T cell clones‡	Total
AL	DRw6cI, DRw11.2	7/8	
(DRw6cI, DRw6cI)	DRw6cI	6/13	
			13/21
BR	DRw6cI, DRw11.1, DRw11.2	2/2	
(DRw6cI, DR4w4)	DRw6cI, DRw11.1	1/1	
	DRw6cI, DRw11.2	0/1	
	DRw6cI	1/4	
	DR4w4	4/8	
			8/16
GC			
(DRw11.1, DRw11.1)	DRw11.1		16/21
KK			
(DR11.1, DR3)	DR11.1		0/1
GAR			
(DRw11.1, DR1)	DRw11.1		0/1
BDM			
(DR11.1, DRw11.1)	DRw11.1		0/1

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 (DRw6cI 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, DRw6cI-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 Vβ, 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., VαJα 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 Vβ, suggesting that the expression of two α chains is likely to be due to

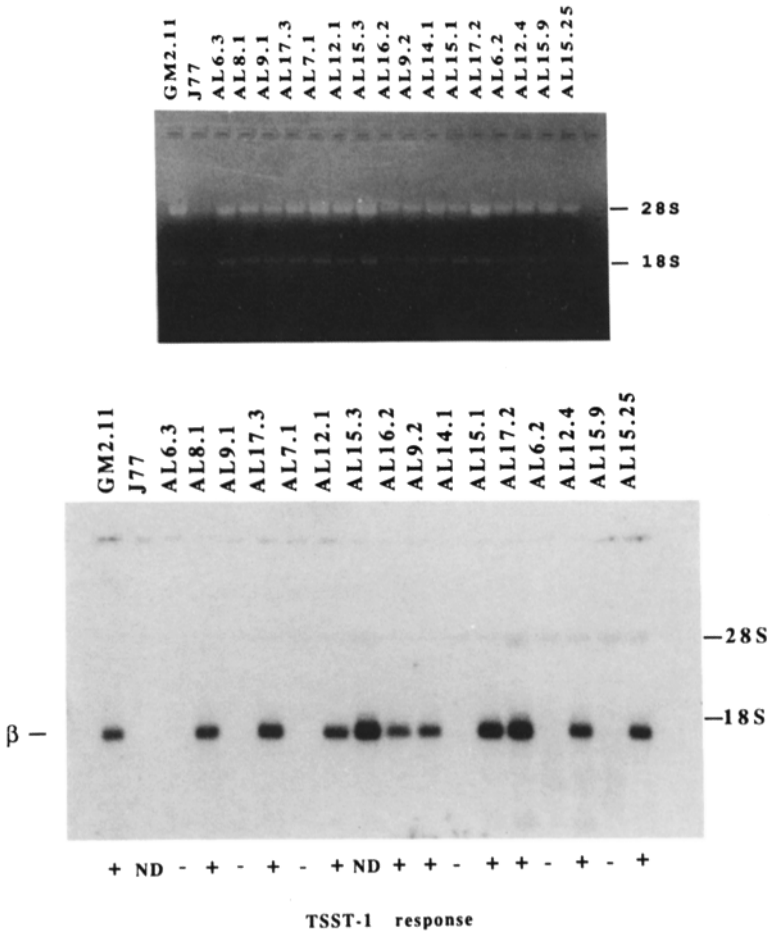

Figure 1. Correlation between Vβ2.1 mRNA expression in AL clones and their proliferative response to the TSST-1. Total RNA (~2.5 μg), isolated from the indicated clones, was stained with ethidium bromide to assess the integrity of the 28S and 18S rRNA (*top*), and subjected to Northern blot analysis (*bottom*). Hybridization was performed with a Vβ2.1 probe isolated from clone GM2.11 (20) (see Materials and Methods). RNA from GM2.11 was used as a positive control. The negative control was RNA isolated from the J77 Jurkat cell line, which expresses a Vβ8 gene (37). The positions of 28S and 18S rRNA are indicated on the autoradiogram (*lower*). Hybridization with the Vβ2.1 probe gives a band of the expected size (~1.3 kb) for TCR-β mRNA. Under each lane is indicated whether the clone responded (+) or not (−) to TSST-1 at concentrations of 0.1–50 ng/ml. Clones were considered positive when the level of [^{3}H]thymidine incorporation was above 10^4 cpm over a background of 10^3 cpm. Most of the clones responding to TSST-1 gave a maximal incorporation between 5×10^4 and 2×10^5 cpm. Clones nonresponding to TSST-1 gave values equal to negative control (without TSST-1). In none of the reported experiments was the background above 10^3 cpm.

lack of allelic exclusion at the level of α rearrangement (28), rather than to contamination by a second T cell clone. In the remaining clones, a single Vα could be detected in 5–10 independent cDNAs sequenced. It is of interest that, besides the unique human Cα sequence described (43), we found a variant in individual AL in which Asn 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 Cα, because in the AL donor, both forms were found in different clones and, when two α 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 α chain rearrangement.

As shown in Table 2, clones from donor AL preferentially used the Vα21.1 and Vα17.1 family members, each in at least 3 and potentially 4 of 10 clones, whereas in the BR donor, two members of the Vα2 family, Vα2.2, and 2.6, are expressed in 4 (or 5) of 10 clones. Thus, individuals AL and BR display similar high frequencies of Vβ2.1 use (≥50%), and their profile of Vα use is more heterogeneous and shows considerably less overlap. Some shared use of Vα gene segments, however, does occur, in that Vα1 family members are expressed in clones AL9.2, BR7.3, and BR22.5, and Vα8 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 Vα family (Vα30), a new member of the Vα14 family (Vα14.1), and a new Jα gene segment (AL 6.3) were identified (Fig. 3).

Multiple Combinations of Vα and Vβ Gene Segments Are Found to Constitute TCRs Specific for tt830–844. As shown in Table 2, a rather diversified repertoire of Vα germline gene segments coexpressed with Vβ2.1 was evident, although repeated use of the same or a related Vα was found in a number of cases. Thus, in donor AL, Vα21.1 was expressed in clones AL15.1 and ALIII6.1, and Vα17.1 was found in clones ALIII4.3, AL17.1, and AL4.1, although in the latter clone Vα14.2 could also potentially pair with Vβ2.1. In donor BR, two Vα2 members, Vα2.2 and Vα2.6, which are 72% identical at the amino acid level, were found associated with Vβ2.1 in two and, potentially three, DRw6cI-restricted clones. In both donors, two additional Vα gene families, Vα8 and Vα1, could also pair with Vβ2.1. Altogether, at least seven different Vα gene segments derived from five distinct families can encode α chains able to pair with Vβ2.1 chains to form a tt830–844/DR-specific binding site. Of note, however, is that Vα21.1, Vα17.1, Vα2.2, and Vα8.1 (Vα8.2), among the Vα segments found in association with Vβ2.1, 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*

Clone T	HLA-DR Restriction	Gene Segments*			
		Vα	Jα	Vβ	Jβ
AL 15.3	DRw6cI P1	Vα21.1	JαR‡	Vβ2.1a	Jβ1.2
ALIII6.1	DRw6cI	Vα21.1	JαU‡	Vβ2.1c	Jβ1.1
ALIII4.3	DRw6cI	Vα17.1	JαAF211‡	Vβ2.1	Jβ2.1
AL 17.3	DRw6cI	Vα17.1	JαF‡	Vβ2.1c	Jβ2.7
AL 4.1	DRw6cI P1	Vα17.1	JαAC9	Vβ2.1a	Jβ1.5
		Vα14.2§	JαAA17		
AL 8.1	DRw6cI P1	Vα8.1	JαIGRJa06	Vβ2.1c	Jβ2.7
AL 12.1					
AL 9.2	DRw6cI	Vα1.10	JαAA17	Vβ2.1c	Jβ2.1‖
ALIII3.1	DRw6cI	Vα21.1	JαIGRJa10	Vβ21.3	Jβ1.5
ALIII6.3	DRw6cI	Vα18.1	JαAL-6.3§	Vβ6.9	Jβ2.5
		Vα21.1	JαG		
AL 7.1	DRw6cI P1	Vα17.1	JαQ‡	Vβ19.1	Jβ2.7
BR 2.2	DRw6cI P2	ND		Vβ2.1c	Jβ1.2
BR 7.5	DRw6cI P3	Vα8.2	JαK	Vβ2.1a	Jβ2.5
		Vα2.6	JαIGRJa10		
BR 9.13	DRw6cI P2	Vα2.6	JαR‡	Vβ2.1c	Jβ2.5
BR 15.3	DRw6cI	Vα2.2	JαAF211	Vβ2.1c	Jβ2.7
BR 7.3	DR4w4	Vα1.2	JαAC17	Vβ2.1a	Jβ2.5
BR 22.5	DR4w4	Vα1.2	JαU	Vβ2.1c	Jβ2.4
BR 1.7	DR4w4	Vα8.1	JαS	Vβ2.1c	Jβ2.3
BR 3.5	DR4w4	Vα2.6	JαIGRJa06‡	Vβ8.3	Jβ2.5
BR 5.11	DR4w4	Vα2.2	JαG	Vβ13.6§	Jβ2.3‡
BR 1.3	DR4w4	Vα6.1	JαH	Vβ7.2	Jβ1.3
KT 2	DRw11.1	Vα30.1§	JαIGRJa13	Vβ12.3	Jβ2.2
GAR 9.2	DRw11.1	Vα8.2	JαS	Vβ19.1	Jβ1.2
		Vα2	JαAA13		

P Indicates promiscuous clones which, in addition to autologous DR recognize tt830–844 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 (AF110), Vα2.6 (AA25), Vα6.1 (HAVP01), Vα8.1 (HAVP41), Vα8.2 (HAVP50), Vα17.1 (AB11), Vα18.1 (AB21), and 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 (HBVT02). Vβ21.3 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, JαAL-6.3, and Vβ13.6 (see Fig. 3).

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

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

	Vα		Jα	Cα		Vβ		Jβ	Cβ
			CDR3					CDR3	
AL15.3	Vα21.1-JαR	CAG	SYNARL	MFGDGTQLVVKP NIQK	Vβ2.1a	-Jβ1.2	CSA	LPRGYYGY	TFGSGTRLTVV EDL
ALIII6.1	Vα21.1-JαU	CAA	SGGQF	YFGTGTSLTVIP NIQ	Vβ2.1c	-Jβ1.1	CSA	RSDPATSA	FFGQGTRLTVV EDL
ALIII4.3	Vα17.1-JαAF211	CAT	LYGQNF	VFGPGTRLSVLP YIQK	Vβ2.1	-Jβ2.1	CSA	RDPGGQAGFYNEQ	FFGPGTRLTVL EDL
AL17.3	Vα17.1-JαF	CAA	SSGNTPL	VFGKGTRLSVIA NIQN	Vβ2.1c	-Jβ2.7	CSA	RASPTYEQ	YFGPGTRLTVT EDL
AL4.1	Vα17.1-JαAC9	CAA	RQGGSEKL	VFGKGTKLTVNP YIQN	Vβ2.1a	-Jβ1.5	CSA	RGGGRPQ	HFGDGTRLSIL EDL
	Vα14.2-JαAA17	CAS	RTGTASKL	TFGTGTRLQVTL DIQK					
AL8.1	Vα8.1 -JαIGRJa06	CAA	ENYGGSQGNL	IFPKGTKLSVKG NIQ	Vβ2.1c	-Jβ2.7	CSA	KTGTSRYEQ	YFGPGTRLTVT EDL
AL9.2	Vα1.10-JαAA17	CVV	SADTGTASKL	TFGTGTRLQVTL DIQK	Vβ2.1c	-Jβ2.1	CSA	RGLPGTSGVSSYNEQ	FFGPGTRLTVL EDL
ALIII3.1	Vα21.1-JαIGRJa10	CAA	RTYNNNDM	RFGAGTRLTVKP NIQ	Vβ21.3	-Jβ1.5	CAS	SFDRVQPQ	HFGDGTRLSIL EDL
AL7.1	Vα17.1-JαQ	CAA	SSRDRGSTLGRL	YFGRGTQLTVWP DIQN	Vβ19.1	-Jβ2.7	CAS	SITGRQ	YFGPGTRLTVT EDL
ALIII6.3	Vα18.1-JαALP2	CAF	SGYSTL	TFGKGTMLLVSP DIQK	Vβ6.9	-Jβ2.5	CAS	TQTLAGREETQ	YFGPGTRLLVL EDL
	Vα21.1-JαG	CAA	RDGSGNTGKL	IFGQGTTLQVKP DIQN					
BR9.13	Vα2.6 -JαR	CVV	NPNNARL	MFGDGTQLVVKP NIQN	Vβ2.1c	-Jβ2.5	CSA	LGLNQETQ	YFGPGTRLLVL EDL
BR15.3	Vα2.2 -JαAF211	CAV	NMYDYGQNF	VFGPGTRLSVLP YIQN	Vβ2.1c	-Jβ2.7	CSA	RDDSGLARASGTSYEQ	YFGPGTRLTVT EDL
BR7.5	Vα2.6 -JαIGRJa10	CVV	AWKDM	RFGAGTRPTVKP NIQ	Vβ2.1a	-Jβ2.5	CSA	SLPGLAIEETQ	YFGPGTRLLVL EDL
	Vα8.2 -JαK	CAE	NSGGSNYKL	TFGKGTLLTVNP NIQN					
BR2.2					Vβ2.1c	-Jβ1.2	CSA	RGNYGY	TFGSGTRLTVV EDL
BR7.3	Vα1.2 -JαAC17	CAV	SDGGAGNML	TFGGGTRLMVKP HIQN	Vβ2.1a	-Jβ2.5	CSA	SSGIEGETQ	YFGPGTRLLVL EDL
BR22.5	Vα1.2 -JαU	CAV	SDPGAGNQF	YFGTGTSLTVIP NIQN	Vβ2.1c	-Jβ2.4	CSA	TSGDKNIQ	YFGAGTRLSVL EDL
BR1.7	Vα8.1 -JαS	CAA	EGPAGTAL	IFGKGTTLSVSS NIQ	Vβ2.1c	-Jβ2.3	CSA	PRGSGLTDTQ	YFGPGTRLTVL EDL
BR3.5	Vα2.6 -JαIGRJa06	CVV	TLPNYGGSQGNL	IFGKGTKLSVKP NIQN	Vβ8.3	-Jβ2.5	CAS	GFRDLGTQ	YFGPGTRLLVL EDL
BR5.11	Vα2.2 -JαG	CAV	TIGSSNTGKL	IFGQGTTLQVKP DIQN	Vβ13.5	-Jβ2.3	CAS	LADSTDTQ	YFGPGTRLTVL EDL
BR1.3	Vα6.1 -JαH	CAM	REVSNNQGGKL	IFGQGTELSVKP NIQN	Vβ7.2	-Jβ1.3	CAS	SQDEGARNTI	YFGEGSWLTVV EDL
KT2	Vα30.1-JαIGRJA13	CAG	DPGNNRKL	IWGLGTSLAVNP NIQN	Vβ12.3	-Jβ2.2	CAI	SNDLLRNTGEL	FFGEGSRLTVL EDL
GAR9.2	Vα8.2 -JαS	CAE	MYQAGTAL	IFGKGTTLSVSS NIQN	Vβ19.1	-Jβ1.2	CAS	SIGQGMRSYGY	TFGSGTRLTVV EDL
	Vα2 -JαAA13	CAM	SAPGNEKL	TFGTGTRLTIIP NIQN					

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.

illustrated by the following examples. Gene segments Vα21 and Vα17 were found to be used not only in association with Vβ2.1, but with Vβ21.3 (clone ALIII3.1) and Vβ19.1 (clone AL7.1), respectively. Similarly, in the TCRs of BR3.5 and BR5.11, Vα2 family members found associated with Vβ2.1 in DRw6cI-restricted clones could pair with other Vβs to generate, this time, a DR4w4-restricted specificity.

Thus, despite the frequent use of Vβ2.1 and of some Vα segments, a large number of Vα/Vβ combinations can constitute TCRs recognizing the tt830–844 peptide, even when considering clones specific for this peptide presented in the same HLA-DR context. Indeed, nine and five different Vα/Vβ pairs were found among 13 DRw6cI-restricted and six DR4w4-restricted clones, respectively.

Apparent Lack of Structural Conservation in the Junctional Regions of anti-tt830–844 TCRs. Many different Jα and Jβ segments were found to be used by the TCRs analyzed (Table 2). We did not notice any particular selection of Jα and Jβ sequences that correlated with DR restriction or with the use of the same Vα and/or Vβ segments. Jβ2.5 and Jβ2.7 were found to be expressed in four and five clones, respectively, and were the most frequently used Jβ segments. However, they could be associated with Vβ2.1, as well as with other Vβ segments. Jα use was more diverse, with a total of 16 different Jα segments expressed in the 23 anti-tt830–844 clones analyzed. This may simply reflect the much greater size of the Jα versus Jβ repertoire (3), rather than selection of particular Jβ segments in the anti-tt830–844 response. Although inspection of the nucleotide sequences of the Vβ-D-Jβ junctions did not always allow a clear assignment of sequences to Dβ1 or Dβ2 segments (40), the contribution of D sequences to the junctional diversity was clearly different from one clone to another.

The large J repertoire and additional junctional variability due to imprecise joining and N (nucleotides)-region addition was reflected in the highly heterogeneous CDR3 sequences of both Vα and Vβ regions (Fig. 2). We assigned the boundaries of the putative TCR CDR3 regions according to Chothia et al. (5). The residues from J sequences contributing to CDR3 are underlined in Fig. 2. The last position of the putative Vα and Vβ CDR3 loops, encoded by the J sequence, is often a Leu and a Gln, respectively. As these residues are normally conserved in the respective J segments (37, 40–42), their role is probably to help maintain the overall conformation of the CDR3 loops, rather than to directly contact ligand (4, 5). The first position of the CDR3 equivalent of Vβ2.1 chains was an Arg in 7 of 14 cases. This residue was germline encoded in all cases except for one, where it

A Vα30

```
                                LEADER
 M  L  L  I  T  S  M  L  V  L  W  M  Q  L  S  Q  V  N  G  Q
ATGCTACTCATCACATCAATGTTGGTCTTATGGATGCAATTGTCACAGGTGAATGGACAA
        10         20         30         40         50         60
 Q  V  M  Q  I  P  Q  Y  Q  H  V  Q  E  G  E  D  F  T  T  Y
CAGGTAATGCAAATTCCTCAGTACCAGCATGTACAAGAAGGAGAGGACTTCACCACGTAC
        70         80         90        100        110        120
 C  N  S  S  T  T  L  S  N  I  Q  W  Y  K  Q  R  P  G  G  H
TGCAATTCCTCAACTACTTTAAGCAATATACAGTGGTATAAGCAAAGGCCTGGTGGACAT
       130        140        150        160        170        180
 P  V  F  L  I  Q  L  V  K  S  G  E  V  K  K  Q  K  R  L  T
CCCGTTTTTTTGATACAGTTAGTGAAGAGTGGAGAAGTGAAGAAGCAGAAAAGACTGACA
       190        200        210        220        230        240
 F  Q  F  G  E  A  K  K  N  S  S  L  H  I  T  A  T  Q  T  T
TTTCAGTTTGGAGAAGCAAAAAAGAACAGCTCCCTGCACATCACAGCCACCCAGACTACA
       250        260        270        280        290        300
 D  V  G  T  Y  F  C  A
GATGTAGGAACCTACTTCTGTGCA
       310        320
```

B Vα14.2

```
                                LEADER
 M  T  R  V  S  L  L  W  A  V  V  V  S  T  C  L  E  S  G  M
ATGACACGAGTTAGCTTGCTGTGGGCAGTCGTGGTCTCCACCTGTCTTGAATCCGGCATG
        10         20         30         40         50         60
___
 A  Q  T  V  T  Q  S  Q  P  E  M  S  V  Q  E  A  E  T  V  T
GCCCAGACAGTCACTCAGTCTCAACCAGAGATGTCTGTGCAGGAGGCAGAGACTGTGACC
        70         80         90        100        110        120
 L  S  C  T  Y  D  T  S  E  N  D  Y  Y  L  F  W  Y  K  Q  P
CTGAGTTGCACATATGACACCAGTGAGAATGATTATTATTTGTTCTGGTACAAGCAGCCT
       130        140        150        160        170        180
 P  S  R  Q  M  I  L  V  I  R  Q  E  A  Y  K  Q  Q  N  A  T
CCCAGCAGGCAGATGATTCTCGTTATTCGCCAAGAAGCTTATAAGCAACAGAATGCAACG
       190        200        210        220        230        240
 E  N  R  F  S  V  N  F  Q  K  A  A  K  S  F  S  L  K  I  S
GAGAATCGTTTCTCTGTGAACTTCCAGAAAGCAGCCAAATCCTTCAGTCTCAAGATCTCA
       250        260        270        280        290        300
 D  S  Q  L  G  D  T  A  M  Y  F  C  A
GACTCACAGCTGGGGGACACTGCGATGTATTTCTGTGCT
       310        320        330        340
```

C Vβ13.6

```
 S  M  T  L  Q  C  T  Q  D  M  N  H  N  Y  M  Y  W  Y  R  Q
GAGCATGACACTGCAGTGTACCCAGGATATGAACCATAACTACATGTACTGGTATCGACA
        10         20         30         40         50         60
 D  P  G  M  G  L  K  L  I  Y  Y  S  V  P  A  G  I  T  D  K
AGACCCAGGCATGGGGCTGAAGCTGATTTATTATTCAGTTCCTGCTGGTATCACTGATAA
        70         80         90        100        110        120
 G  E  V  P  N  G  Y  N  V  S  R  S  T  T  E  D  F  P  L  R
AGGAGAAGTCCCGAATGGCTACAACGTCTCCAGATCAACCACAGAGGATTTCCCGCTCAG
       130        140        150        160        170        180
 L  E  L  A  A  P  S  Q  T  C  L  Y  F  C  A  S
GCTGGAGTTGGCTGCTCCCTCCCAGACATGTCTCTACTTCTGTGCCAGC
       250        260        270        280        290
```

D Jα-AL6.3

```
 F  S  G  Y  S  T  L  T  F  G  K  G  T  M  L  L  V  S  P
TTTTCAGGATACAGCACCCTCACCTTTGGGAAGGGGACTATGCTTCTAGTCTCTCCA
        10         20         30         40         50         60
```

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*) Vα14.2 (from clone AL4.1) and (*C*) Vβ13.6 (from clone BR5.11) correspond to new sequences belonging to already described families. Vα14.2 shares 93.5% nucleotide identity with the HAVT20 prototype (38) of the Vα14 family. Vβ13.6 is a partial sequence but shows 90–96% nucleotide identity with the previously described sequences of the Vβ13 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).

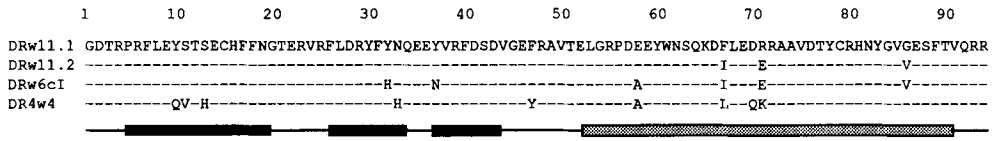

Figure 4. Amino acid sequence of the first domain of the β chain of DRw11.1, DRw11.2, DRw6cI, and DR4w4 alleles. Amino acid residues are indicated using single-letter code and are from reference 59; (*dashes*) identity to the DRw11.1 sequence. Below the sequence is a schematic representation of the proposed secondary structure of DRβ chain; (*black boxes*) β strands, (*grey box*) the α-helix, and (*thin lines*) turns and bends of the polypeptide chain.

arose from somatic diversification mechanisms (not shown). 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 hemaglutinin, 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 DR6wcI, DR4w4, or DRw11.1 and DRw11.2 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 Wucherpfenning 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, tt830–844 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 DRw6cI, 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 antiphenyloxazolone (50) antibodies about how a particular binding specificity may be preserved, even after substitution of a given H (or L) chain by another, apparently unrelated one. In the former case, the H chains are invariably derived from the same V_H germline gene, and the L chain sequences may belong to any of three different subgroups (49). In the latter case, a given H chain may be functionally replaced by a variety of other, seemingly unrelated ones, provided the L chain is retained (50). In both cases, however, key antigen-contacting residues are found to be conserved in the substituted chain. Another possible explanation for the diversity of α chains which may be paired with Vβ2.1-encoded β chains is that recognition is mostly mediated by the β chain and that the α chain plays only a secondary role and so is not functionally selected. In this context, Tan et al. (51) have shown that transfer of the α chain alone of an arsonate-specific TCR into a recipient T cell with an unrelated β chain is sufficient to confer responsiveness to the hapten, implying that one chain of the TCR may be functionally dominant over the other in certain cases.

It is interesting that clones expressing the same or very closely related Vα and the same Vβ segments, but displaying distinct junctional amino acid sequences, could recognize tt830–844 in the context of two different DR alleles. It therefore appears that in some cases TCR junctional regions may dictate the restriction by recognizing either a DR allele–dependent conformation of the peptide or polymorphic residues of the DR β chain. A situation in which TCR junctional regions seem critical to recognition of both antigen and MHC has been described by Rupp et al. (52) who found that an alloreactive, H-2D^b-specific, cytotoxic T cell clone and a chicken erythrocyte-specific, I-A^b–restricted, helper clone share identical Vα and Vβ gene segments, but differ in their junctional sequences. Thus, alterations in the putative CDR3 regions of the TCR may affect peptide specificity but not MHC restriction (15–18), both antigen recognition and MHC restriction (52), or only MHC specificity but not peptide reactivity, as in the present work.

The most striking finding of this study was the remarkable diversity in the junctional regions of the 23 anti-tt830–844 T cell clones analyzed, even when comparing TCRs composed of identical Vα and/or Vβ gene segments. Thus, among 14 anti-tt830–844 T cell clones using the Vβ2.1 gene segment, 14 unique Vβ-D-Jβ junctions were found, with no evident conservation of length and/or amino acid composition, and with only a single positively charged amino acid residue conserved at the NH_2-terminal position of the putative CDR3s of eight of them (Table 2). However, the fact that this residue is not always conserved, as well as being encoded by the germline Vβ2.1 gene segment (9) in seven of the eight cases, argues against any particular selection of this residue for recognition. Lack of structural conservation was also found for most of the putative CDR3s of the α chains, whether or not they were associated with the same Vα gene segment or were part of TCRs using the same Vβ gene segment. One exception was represented by clones BR7.3 and BR22.5, whose α chain CDR3 junctions are partially conserved in length and amino acid composition. Here too, however, one finds the 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 tt830–844/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 V_L-J_L and V_H-D-J_H junctions (CDR3s) observed among anti-phosphorylcholine (49) and anti-phenyloxazolone (50) antibodies, is largely due to structural constraints related to antigen recognition. Conversely, CDRs that make no contacts with antigen, such as V_H CDR2 of anti-phenyloxazolone antibodies (50), are not functionally restrained and may show extensive sequence variability. An interesting finding in this respect is that an L^d-reactive cytolytic T cell clone using the same Vα segment (Vα3) as an I-A^d-restricted arsonate reactive one, but an unrelated Vα-Jα junction and β chain, also responds to this hapten, suggesting that CDR3 sequences may not be critical to arsonate recognition (51).

Alternatively, our data can be interpreted in terms of recently proposed models for TCR recognition of peptide/MHC complexes in which CDR1 and CDR2 of the V regions interact with MHC determinants, and the CDR3 junctional regions bind to the antigenic peptide (3, 5, 56). Assuming that all Vβ2.1-containing TCRs bind to the same tt830–844/DR complex in the same general orientation as the result of conserved contacts between the first two CDRs of this domain and the DR molecule, one is confronted with the obvious difficulty of physically accommodating such highly heterogeneous CDR3s in what is predicted to be the geometrical center of the interface between the TCR and the peptide/MHC complex (3, 5, 56). This problem is even more difficult for TCRs using the same Vα and Vβ segments. One possibility is that the Vβ2.1 CDR3s and Vα CDR3s differing dramatically in primary structure, may actually fold into similar three-dimensional conformations. This, however, appears unlikely based on the similarity between Ig and TCR frameworks (5) and on detailed studies of the conformations of V_L and V_H CDR3s in antibodies of known three-dimensional structure (53). An alternative hypothesis to explain CDR3 heterogeneity in TCRs recognizing the same complex is that certain peptides bound to the MHC pocket possess a degree of freedom allowing some of the peptide side chains to assume different conformations (17). This in turn may result in different complexes selecting the same V regions (which contact the MHC molecule), but very different junctions.

It is also possible that even TCRs using the same Vβ (and Vα) germline gene segments may have different views of the same peptide/DR complex or see different parts of tt830–844, thus explaining the junctional variability observed. However, preliminary studies examining the effects of multiple substitutions at every position of tt830–844 on recognition by some of the Vβ2.1-expressing clones reported here indicate that the segment seen by these clones does not include the NH_2-terminal (Q) and the last four COOH-terminal (ITEL) residues of tt830–844. 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 V_L versus V_H 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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