T Cell Receptor Selection by and Recognition of Two Class I Major Histocompatibility Complex–restricted Antigenic Peptides That Differ at a Single Position

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Summary

Peptides derived from HLA-Cw3 and HLA-A24 within region 170–179 differ by a single substitution, at position 173, and are both presented by the class I major histocompatibility complex molecule H-2K^d for recognition by murine cytolytic T lymphocytes (CTLs). As a first approach to understand the way T cell receptors (TCRs) intact with the HLA peptides, we have analyzed the TCR selection by, and recognition of, the two HLA antigenic sites. First, we have compared the TCR repertoires selected by HLA-Cw3 and HLA-A24, not only by sequencing the TCRs carried by CTL clones isolated and grown in vitro, but also by analyzing the TCRs expressed in vivo by peritoneal exudate lymphocytes from immune animals. Second, we have compared the TCR crossrecognition of HLA-A24 by CTLs selected by HLA-Cw3 with that of HLA-Cw3 by CTLs selected by HLA-A24. The combined analysis of TCR selection by and recognition of these two related HLA antigenic sites provides evidence that the TCR β junctional regions interact with the amino-terminal part of the HLA peptides.

CTLs recognize antigenic peptides presented by class I MHC molecules (1, 2). The specificity of this recognition is conferred by the TCR α/β (3, 4). Whereas the structure of several class I MHC molecules, and that of a class I MHC-peptide complex, were determined by crystallographic studies (5), such information is not available yet for the TCR. However, a model of the TCR α/β tertiary structure was proposed, based on its homology with Igs (6). In any case, experimental evidence is lacking so far to support a topology of class I MHC-peptide recognition by TCRs.

We previously reported that DBA/2 mice could mount a CTL response towards two related antigens, HLA-Cw3 and HLA-A24, in the context of the same murine class I MHC molecule, H-2K^d, and that a fraction of the CTLs raised in response to either HLA antigen was not reactive to the second HLA antigen (7). The optimal synthetic peptides recognized by these specific CTLs, corresponding to the region 170–179, differ by a single nonconservative substitution at position 173 and bind their common restriction element H-2K^d with a similar affinity (Table 1) (8–12). Regions 170–179 of the related HLA molecules A2 and A3 are identical to HLA-A24 and can also be recognized by H-2K^d-restricted CTLs (13).

We recently showed that the TCRs carried by H-2K^d-restricted CTLs specific for the Cw3 170–179 peptide were very limited in primary structure (14). They were encoded by few germline gene segments: a single Vβ segment (Vβ10), a single Jα segment (JαpHDS58), few Vα segments (mainly Vα3, 4, 8), and few Jβ segments (mainly Jβ1.2, 1.4, 2.3). Their junctional CDR3 α and β loops also displayed limited diversity: a single length of nine and six amino acids, respectively, a conserved non-V-, non-J-encoded glycine amino acid at position 97 in the CDR3 β, and a high occurrence of non-J-encoded glycine or charged amino acids at positions 94 and 95 in the CDR3 α.

As a first approach to understand the topology of interaction of TCRs with their class I MHC-peptide ligand, we have now taken advantage both of the single amino acid difference between the Cw3 and A24 antigenic peptides and of the existence within each of the two CTL responses to HLA antigens of a noncrossreactive CTL population.

We first tested a large series of Cw3-selected CTL clones bearing TCRs of known primary structure for crossrecognition of A24. We then determined the TCR repertoire selected by the A24 peptide, not only by sequencing the TCRs carried by CTL clones isolated and grown in vitro, but also by analyzing the TCR repertoire used in vivo by peritoneal ex-

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Table 1. *HLA-Cw3 and HLA-A24 170–179 Antigenic Peptides*

Cw3	R	Y	L	K	N	G	K	E	T	L
A24	–	–	–	E	–	–	–	–	–	–

The HLA-Cw3 and -A24 molecules differ at a single position (173) within region 170–179 (8). The HLA-A2 and -A3 molecules, as well as the recombinant molecules 2.2/3.3 and 3.3/2.3, display the same amino acid sequence as HLA-A24 (13). Both peptides harbor the K^d binding motif (underlined) (2, 11, 12) and bind equally well to K^d (not shown).

udate lymphocytes (PELs)[1] from immune animals. Finally, we tested some of these A24-seleced CTL clones for cross-recognition of Cw3. Our findings provide evidence that the TCR β junctional regions interact with the amino-terminal part of the class I MHC-restricted HLA antigenic peptides.

Materials and Methods

CTL Clones and PELs. The CTL clones used in this study are listed in Table 2. They were maintained in culture as described in the references. PELs were isolated and analyzed as previously described (14).

Cell Staining and Cytolytic Assays. The cell staining was performed as previously described, as were the cytolytic assays (14).

Direct Sequencing of PCR Products. The cDNA PCR and direct sequencing were performed as previously described (14–17).

Oligonucleotides. The Jβ1.2 and Jβ1.4 primers will be reported elsewhere (Pannetier et al., manuscript in preparation). Sequences of the newly designed primers specific for the VαT2.5–5 (18) and VαB6.2.16 subfamilies (19) are CAGAAAACAGAGCCAAAGAC and GAGACACCGTTGTTAAAGGC, respectively. The other primers were previously described (15).

Results

Crossrecognition of A24 by CTL Clones Selected by Cw3. We previously reported the TCR sequences of 37 independent H-2K^d-restricted CTL clones specific for the Cw3 170–179 antigenic site (14; Casanova et al., manuscript in preparation). We have now tested 30 of them for crossrecognition of the same antigenic site within A24.

As summarized in Fig. 1, where the TCR primary structures are shown along with the peptide fine specificity, most anti-Cw3 CTL clones are affected by the substitution at position 173. 11 clones do not recognize A24 at all, and 16 clones display an "intermediate" recognition profile of A24. Among the latter category, the patterns were in fact heterogeneous, as illustrated in Fig. 2. Only three clones appear to recognize A24 as well as Cw3.

The usage of the only two variable segments of the TCR repertoire selected by Cw3, namely Jβ and Vα, was not evenly distributed between crossreactive and noncrossreactive clones. The Jβ1.4 and Jβ2.3 segments were more frequent among noncrossreactive (6/11) than crossreactive (3/19) clones, whereas the reverse trend was found for Jβ1.2. The Vα3 subfamily was more frequent among noncrossreactive (5/11) than crossreactive (1/19) clones, whereas the reverse trend was found for Vα4 and Vα8.

The amino acids occurring at two positions of the CDR3 α also showed some bias with respect to crossreactivity pattern. At position 95, many (11/19) crossreactive clones expressed a non-V-, non-J-encoded positively charged residue, whereas none of the 11 noncrossreactive ones did. The frequency of negatively charged residues at positions 94 in the CDR3 α, mostly Vα4 and Vα8 encoded, was higher among crossreactive (16/19) than noncrossreactive (6/11) CTL clones.

An examination of the reactivity patterns of individual clones that share identical α or β chains further illustrates this uneven distribution of TCR structural features, and demonstrates that the pattern of A24 crossreaction cannot be attributed solely to either chain. Several sets of CTL clones displaying identical TCR β chains, but different reactivities, were found. Clones Cw3/4A3, HLA1G6, PEA1, and PEA34 crossreact with A24, unlike PEA13, although they share the same TCR β sequence. Notably, the former clones are all Vα8 and their CDR3 α contain a pair of complementary charges, while the latter clone is Vα3 and its CDR α contains only an acid charge. Similarly, clones Cw3/PEA30 and HLA1C8 share the same TCR β sequence and are both Vα4, but the former does crossreact and its CDR3α contains a pair of opposite charges, whereas the latter does not crossreact and its CDR3α only contains an acid residue. Finally, whereas the clone Cw3/C37 is Vα8, with a CDR3 α containing two complementary charges, the clone HLA2D3 is Vα4, its CDR3 α contains no charged residue, and only the former clone crossreacts although they both display the same TCR β sequence.

Conversely, CTL clones sharing an identical TCR α chain (although, rigorously, divergence in the Vα segment upstream of the region sequenced may exist), but differing in fine specificity, were also found. Clones Cw3/1.1, HLA2A3, and PEA21 express the same TCR α chain, but only the latter does not crossreact with A24. Its TCR β differs from that of the two other clones in Jβ segment usage (Jβ2.3 instead of Jβ1.2 or Jβ2.7).

Thus, in the absence of any strict structure-function segregation, it is impossible to conclude from these data that any of these TCR regions confer or block crossrecognition of A24. Rather, it appears that crossrecognition of A24 does not depend on a single region of the TCR, since several regions of the α/β heterodimer correlate with the crossreactivity patterns. Moreover, the possibility remains that any of these TCR regions may contribute to the crossreaction pattern by modulating the overall affinity of the complex, even by interacting with a region distinct from residue 173. Altogether, a firm conclusion in terms of topology of the ternary complex cannot be drawn from these first results alone. To investigate further the contribution of each of these TCR elements, we next assessed the effect of the substitution at position 173 found in A24 on the direct selection of a TCR repertoire.

[1] *Abbreviation used in this paper:* PEL, peritoneal exudate lymphocyte.

Table 2. *Origin of H-2K^d-restricted CTL Clones Selected by the HLA-A24 170–179 Antigenic Site Used in This Study*

Mouse	Strain	Immunogen*	In vitro stimulation	CTL clones	Reference
1	DBA/2	P815-A24	P815-A24	A24/10.1, 12.2	7
2	DBA/2	P815-A3	P815-A3	A3/74.1, 72.2	10
3	DBA/2	P815-A3/hβ2m	P815-A3/hβ2m	A3/IC1	This report‡
4	DBA/2	P815-A3/hβ2m	P815-A3/hβ2m	A3/IIC7	This report‡
5	DBA/2	P815-A3/hβ2m	P815-A3/hβ2m	A3/IIIC5	This report‡
6	F_1(B10.D2 × LG/Ckc)	P815-A3/hβ2m	P815-A3/hβ2m	A3/H2R2, H2R5	This report‡
7	F_1(B10.D2 × LG/Ckc)	P815-A3/hβ2m	P815-A3/hβ2m	A3/C32b, C46b, C80b	This report‡
8	DBA/2	P815-A2	P815-A2	A2/25	13
9	DBA/2	P815-A3	P815-A3	A3/63	13
10	DBA/2	P815-2.2/3.2	P815-2.2/3.2	223/5, 14, 27	13
11	DBA/2	P815-3.3/2.3	P815-3.3/2.3	332/1K, 2A, 2G	13
12	DBA/2	P815-A24	P815-A24	A24/PEF1, 2, 4, 5, 8	This report§
13	DBA/2	P815-A24	P815-A24	A24/PEG2	This report§

The specificity of the CTL clones from mice 1, 2, and 8–13 was established by recognition of the transfectant cell line P815-A24 (6) and was further documented with P815 cells pulsed with synthetic peptides corresponding to the region 170–182 (for clones A24/12.2 and A3/74.1) or 170–179 (all other clones) of the HLA-A24 molecule. The specificity of CTL clones from mice 3–7 was established by recognition of P815 cells transfected with HLA-A3, whose sequence in the 170–179 region is identical to that of HLA-A24. The H-2K^d restriction of the CTL clones mice 3–7 is based on antibody blocking experiments, and that of clones from mice 1, 2, and 8–13 is presumed from recognition of A24 peptides, which are known to bind to H-2K^d.

* P815-A24 indicates a P815 mastocytoma cell line transfected with the HLA-A24 gene (6), and P815-A2, A3, 2.2/3.2, and 3.3/2.3 indicate P815 cell lines transfected with natural or recombinant genes encoding proteins that share A24 sequence within region 170–179 (13). P185-A3/hβ2m indicates a P185 cell line transfected with the HLA-A3 gene and the human β2m gene (Barra et al., unpublished results).

‡ These clones were isolated as described in references 6 and 10.

§ These clones were derived by limiting dilution of CD8$^+$ PELs, from animals immunized intraperitoneally with P815-A24 transfectant cells.

TCRs Carried by CTL Clones Selected by A24. We analyzed a collection of 26 H-2K^d-restricted CTL clones specific for the site 170–179 of HLA-A24 that were judged to be independent based on differences either in TCR nucleotide sequence or in the animal of origin (Table 2).

The TCR repertoire selected by A24 appears to be in many respects similar to the TCR repertoire selected by Cw3 (Figs. 3 and 4). In particular, most CTL clones express TCRs bearing the Vβ10 and JαpHDS58 segments. However, the A24-selected TCR repertoire also seems to be broader, as illustrated by the presence of additional structures in most regions of the α/β heterodimers.

When precisely compared with the TCRs from the Cw3-selected TCR repertoire, the TCRs selected by A24 clearly fall in three categories. The first group (I) includes 11 TCRs that are indistinguishable from those found in the Cw3-selected TCR repertoire. They express the Vβ10 segment, a Jβ1.2, 2.3, 1.1, or 2.7 segment, a Vα8, 4, 3, or 5 segment, and the JαpHDS58 segment. They display a CDR3 α length of nine amino acids, a CDR3 β length of six amino acids, a glycine or a charged residue at positions 94 and 95 in the CDR3 α, and a glycine at position 97 in the CDR3 β. The resemblance with the HLA-Cw3 TCR repertoire is such that six and four of these A24-selected CTL clones express TCR β and α chains, respectively, identical to some of those found among Cw3-selected CTL clones. The other TCRs differ from the Cw3-selected TCRs either by few conservative substitutions in either of the CDR3 or by the usage of a distinct member of the same Vα subfamily.

The second group (II) includes six TCRs, which differ from the CW3-reactive TCRs by only a single criterion. Five clones express a CDR3 β length of 10 amino acids, all of which are Vβ10-Jβ1.4. In contrast, all Vβ10-Jβ1.4 CDR3 loops in the anti-Cw3 TCR repertoire were of six amino acids. In addition, one clone uses the Jβ2.4 segment, which was not found in the Cw3-reactive TCR repertoire. Among the TCRs of group II, one TCR α chain is identical to one found in the Cw3 repertoire, and the others differ by only a few conservative substitutions in the CDR3 or by the usage of a distinct member of the same Vα subfamily from the anti-Cw3 TCR repertoire.

The third group (III) includes nine clones, in which at least two changes were observed when compared with the TCR features characteristic of the response to Cw3. Remarkably, in all cases at least one of these changes concerns the TCR β junctional region, again either in the usage of different Jβ segments (Jβ2.4, 1.3, 2.5, 2.1), or of different CDR3 lengths (Vβ10-Jβ1.4 loops of 10 amino acids). Additional changes can affect every part of the heterodimers: Vβ segment usage, CDR3 β length, lack of glycine at position 97 in the CDR3 β, Vα subfamilies usage, CDR3 α length, and Jα segment usage.

CTL clone	TCR β					TCR α					REACTIVITY	
	Vβ	FW	CDR3	FW	Jβ	Vα	FW	CDR3	FW	Jα	CW3	A24
			99					94				
Cw3/1.1	10	CAS	S L G S D Y	TFG	1.2	8.p71	CAL	S E G G F A S A L	TFG	pHDS58		
Cw3/Cas20	10	CAS	S L G S D Y	TFG	1.2	8.p71	CAL	S D Q G F A S A L	TFG	pHDS58		
Cw3/2C1	10	CAS	S L G S D Y	TFG	1.2	4.TA65	CAL	S D R G F A S A L	TFG	pHDS58		
Cw3/PED5	10	CAS	S L G S D Y	TFG	1.2	8.F3.6	CAL	S D Q G F A S A L	TFG	pHDS58		
Cw3/PEA14	10	CAS	S Y G S D Y	TFG	1.2	8.p71	CAL	S D R G F A S A L	TFG	pHDS58		
Cw3/PEA23	10	CAS	S Y G S D Y	TFG	1.2	4.PJR-25	CAL	S D R G F A S A L	TFG	pHDS58		
Cw3/Cas2	10	CAS	S F G S D Y	TFG	1.2	8.F3.2	CAL	S D R G F A S A L	TFG	pHDS58		
Cw3/4A3	10	CAS	S R G S D Y	TFG	1.2	4.PJR-25	CAL	S D R G F A S A L	TFG	pHDS58		
Cw3/HLA1G6	10	CAS	S R G S D Y	TFG	1.2	4.1G6	CAL	S D R G F A S A L	TFG	pHDS58		
Cw3/PEA1	10	CAS	S R G S D Y	TFG	1.2	4.PJR-25	CAL	S D R G F A S A L	TFG	pHDS58		
Cw3/PEA13	10	CAS	S R G S D Y	TFG	1.2	8.F3.6	CAL	S D Q G F A S A L	TFG	pHDS58		
Cw3/PEA34	10	CAS	S R G S D Y	TFG	1.2	4.PJR-25	CAL	S D R G F A S A L	TFG	pHDS58		
Cw3/C37	10	CAS	S Y G T D Y	TFG	1.2	8.F3.6	CAL	S D R G F A S A L	TFG	pHDS58		
Cw3/HLA2D3	10	CAS	S Y G T D Y	TFG	1.2	3.A8	CAV	S A G G F A S A L	TFG	pHDS58		
Cw3/HLA1C8	10	CAS	S Q G T D Y	TFG	1.2	4.3	CAL	G D P G F A S A L	TFG	pHDS58		
Cw3/PEA30	10	CAS	S Q G T D Y	TFG	1.2	4.PJR-25	CAL	S X G G F A S A L	TFG	pHDS58		
Cw3/10.1	10	CAS	S T G F D Y	TFG	1.2	1.TA84	CAV	S E H G F A S A L	TFG	pHDS58		
Cw3/PEA9	10	CAS	S Q G P D Y	TFG	1.2	4.MD-13	CAL	S D R G F A S A L	TFG	pHDS58		
Cw3/A8	10	CAS	S L G E T L	YFG	2.3	3.A8	CAV	S M G G F A S A L	TFG	pHDS58		
Cw3/Cas1	10	CAS	S L G E T L	YFG	2.3	5.MDA	CAV	S A G G F A S A L	TFG	pHDS58		
Cw3/PEA21	10	CAS	S F G E T L	YFG	2.3	8.p71	CAL	S E G G F A S A L	TFG	pHDS58		
Cw3/701.1	10	CAS	S Y G E T L	YFG	2.3	3.AR-5	CAL	T Q T G F A S A L	TFG	pHDS58		
Cw3/56.1	10	CAS	S W G E T L	YFG	2.3	3.AR-5	CAL	S A T G F A S A L	TFG	pHDS58		
Cw3/2C3	10	CAS	S Y G E R L	FFG	1.4	5.MDA	CAV	S E G G F A S A L	TFG	pHDS58		
Cw3/PED2	10	CAS	S Y G E R L	FFG	1.4	3.AR-5	CAL	S A T G F A S A L	TFG	pHDS58		
Cw3/Cas7	10	CAS	S F G E R L	FFG	1.4	8.F3.2	CAL	S E R G F A S A L	TFG	pHDS58		
Cw3/1B4	10	CAS	S Q G E R L	FFG	1.4	3.AR-5	CAL	S A T G F A S A L	TFG	pHDS58		
Cw3/5B8	10	CAS	S K G V M G	YFG	2.7	4.3	CAL	G E G G F A S A L	TFG	pHDS58		
Cw3/HLA2A3	10	CAS	S S G R V E	YFG	2.7	8.p71	CAL	S E G G F A S A L	TFG	pHDS58		
Cw3/Cas15	10	CAS	S F G Q E V	FFG	1.1	8.p71	CAL	S D G G F A S A L	TFG	pHDS58		

Figure 1. TCR α and β chains carried by Cw3-selected CTL clones and their crossrecognition of A24. The nucleotide sequences and the deduced amino acid sequences of the TCR α and β junctional regions carried by 20 of the 30 anti-Cw3 CTL clones represented here were already published (14). The TCR sequences of the remaining 10 clones (Cw3/PEA, PED), all derived from PELs, will be reported elsewhere with those of additional anti-Cw3 CTL clones also derived from PELs. The Vβ, Vα, Jβ, and Jα segments are represented. The deduced amino acid sequences of the junctional and hypervariable region, putatively CDR3-like, are reported (in single-letter amino acid code) according to reference 6. The presumed Ig-like loop, designated CDR3 for convenience, is putatively supported by two framework branches (FW), which are also reported here. The key Cys residue is at positions 90 and 92 in the α and β chains, respectively. The TCR α junctional region of CTL clone Cw3/PEA30 could not be unambiguously determined. All CTL clones recognize the HLA-Cw3 170–179 antigenic site (□). Levels of crossrecognition of the A24 170–179 antigenic site are indicated: (□) recognition of A24 as well as that of Cw3; (▨) intermediate recognition; or (■) no recognition. Clones Cw3/A8, Cas1, Cas20, 1.1, 10.1, 701.1, PED2, and PED5 were tested for recognition of the Cw3 and A24 170–179 synthetic peptides and for recognition of the P815-Cw3 and A24 transfectant cells. Clone Cw3/56.1 was tested for recognition of P815-Cw3 and A24 transfectant cells. Clones Cw3/PEA1, 9, 13, 14, 21, 23, 30, and 34 were tested for recognition of the HLA-Cw3 and A24 170–179 peptides, and for recognition of the P815-Cw3 and A24 transfectant cells. Clones Cw3/HLA1G6, HLA2D3, HLA1C8, and HLA2A3 were tested for recognition of the HLA-Cw3 and A24 170–179 peptides, and clone HLA2D3 was further tested on P815-Cw3 and A24 transfectant cells. Clones Cw3/Cas2, Cas7, Cas15, C37, 2C1, 4A3, 1B4, 5B8, and 2C3 were tested for recognition of the HLA-Cw3 and A24 peptides. E/T ratios or peptide concentrations were titrated to evaluate the relative recognition of transfectants or peptides, respectively.

When compared with the Cw3-selected TCRs that did not crossrecognize A24, it appears that A24-selected TCRs display paradoxically a similar distribution in terms of Vα3 subfamily usage and charged amino acid composition at positions 94 and 95 of the CDR3 α.

The frequency of Vα3 among A24-selected clones (6/26) is similar to that of Cw3-selected clones (6/30), although of the latter Vα3 clones, most did not crossrecognize A24. The frequency of negatively charged residues in the CDR3 α at position 94 is also lower (8/26 vs. 22/30), and even lower is the frequency of positively charged residues at position 95 (5/26 vs. 12/30), although again the negatively charged residue at position 94, and even more, the positively charged residue at position 95, were found to be less frequent among the anti-Cw3 clones that did not crossrecognize A24.

These results suggest that the Vα usage and the CDR3 α composition in charged residues would not be critical for recognition of position 173 of the HLA peptides, but rather would contact another region, possibly contributing to the crossreaction patterns observed among Cw3-selected clones tested for crossrecognition of A24 by modulating the overall affinity of the TCRs for their ligand.

In contrast, among the A24-selected CTL clones, the usage of Jβ2.3 is reduced (1/26), and that of Vβ10-Jβ1.4 loops of six amino acids is abolished, in agreement with the previous observation that within the Cw3-selected repertoire, their

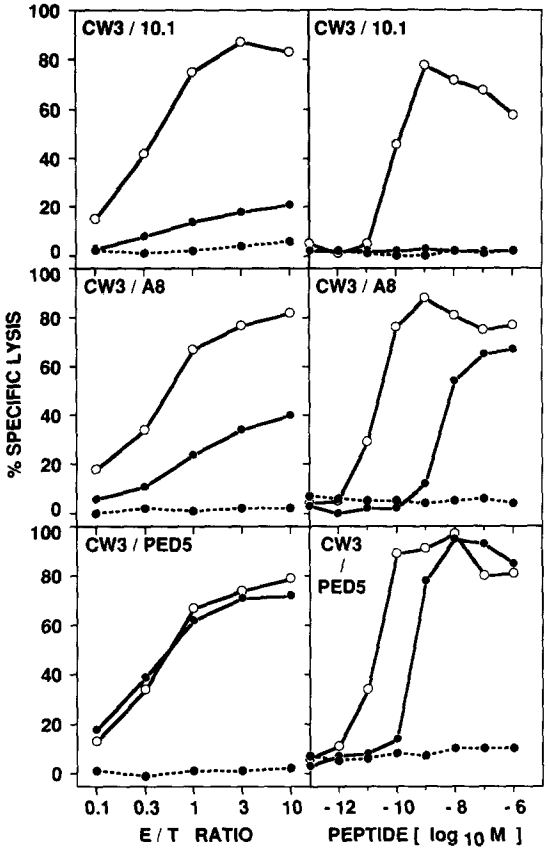

Figure 2. Crossrecognition of A24 by a set of Cw3-selected CTL clones. Three Cw3-selected CTL clones, Cw3/10.1, A8, and PED5, were tested for recognition either of P815 cells transfected with the HLA-Cw3 (O) or A24 (●) genes (*left*), or of P815 (H-2^d) target cells pulsed with HLA-Cw3 (O) or A24 (●) 170–179 peptides (*right*). Lysis of control P815 cells without peptide is also shown (*dashed lines*).

frequency was lower among the clones that did not cross-recognize A24 than among those that did.

These results in turn suggest that the TCR β junctional region is critical for recognition of position 173. Nevertheless, the differences observed in the TCR repertoires selected by Cw3 and A24 may result either from an indirect effect, due to antigen-independent selective processes, or from differences in the antigen-driven selection, directly related to the MHC/antigenic peptide recognition. In an attempt to discriminate between the two hypotheses, we then tested A24-reactive CTL clones of known TCR primary structure for recognition of HLA-Cw3.

Crossrecognition of Cw3 by CTL Clones Selected by A24. We tested 17 independent A24-selected CTL clones for crossrecognition of Cw3. Three clearcut categories emerged from these data, summarized in Fig. 4 and illustrated for two clones in Fig. 5. Four clones recognized Cw3 as well as A24. Two clones displayed an "intermediate pattern" of crossreactivity. 11 clones were found to be completely negative for cross-recognition of the Cw3 antigen.

All clones that crossreact equally well with Cw3 belong to the group I of TCR structures, i.e., the group of TCRs most similar, when not identical, to those expressed by Cw3-reactive CTL clones. The two clones with an intermediate reactivity, A3/74.1 and A24/12.2, belong to groups I and III, respectively. The clone A3/74.1 bears the Jβ1.1 segment and has therefore been classified in group I. However, this segment was found in only 2 of 37 Cw3-selected CTL clones, which may in part explain the somewhat lower crossreactivity of A3/74.1. Among the remaining 11 clones that do not recognize at all the Cw3 sequence, only one belongs to the group I, 223/27, and this CTL clone not only again bears the Jβ1.1 segment, but also does not express any acid residue in the CDR3 due to extensive trimming of the Jβ1.1 segment. Of the remaining 10 clones, four belong to group II and six to group III.

Two CTL clones, 332/1K and A3/63, were found to express an identical TCR chain, and the comparison of their crossreactivity supports our previous conclusions. These two clones bear identical TCR α chains, but whereas 332/1K cross-recognizes Cw3 as well as A24, A3/63 does not crossreact at all.

Thus, in contrast to the lack of strict structure-function correlation previously observed among the Cw3-selected CTL clones tested for crossrecognition of A24, a clear-cut segregation can be seen between the TCR β junction primary structure of A24-selected CTL clones and their crossrecognition of Cw3. The A24-selected CTL clones whose TCRs differ from those carried by Cw3-selected CTL clones do not crossrecognize CW3, and the TCR differences take place at least, and often only, in the TCR β junctional region. Therefore, these data point towards a role of this region for recognition of position 173.

TCRs Expressed In Vivo by PELs from Cw3- and A24-immunized Mice. To rule out any bias in vitro during the isolation of this set of CTL clones, or any bias due to an insufficient number of CTL clones analyzed, we then compared the TCR repertoire used in vivo in response to Cw3 and A24.

PELs were harvested after intraperitoneal immunization with P815-Cw3 and P815-A24 transfectant cells, and analyzed as previously described (14). They displayed specific cytolytic activity without any stimulation in vitro (not shown). The proportion of Vβ10 among CD8 cells was significantly increased, indicating that the Vβ10 dominance observed on CTL clones also occurred in vivo (not shown). However, among A24-selected PELs, the higher variation of the Vβ10 percentage among individuals, as well as the somewhat lower percentage on average than that found among Cw3-selected PELs, were compatible with the finding that clones expressing other Vβ segments may occasionally be selected by A24, as was the Vβ15 CTL clone PEG2, derived from one mouse in which no Vβ10 increase was observed.

To analyze the TCR response in more detail, a first cDNA PCR with a Vβ10 and a Jβ1.2 pair of primers was performed on the Cw3- and A24-specific PELs, and the product was directly sequenced with the Jβ1.2 primer. As shown in Fig. 6, the product was not only clearly readable, indicating a homogeneity of the TCRs bearing these two segments, but also encoded a CDR3 of the same length as that of Cw3- or A24-reactive CTL clones known to express Vβ10-Jβ1.2

A TCR α cDNAs

CTL clone	Vα		Jα
A3/C80b	8.F3.4	...TGT GCT TTG AGT GAT GGG GGC TTT GCA AGT GCG CTG ACA TTT GGA...	pHDS58
A3/IC1	8.F3.4	...TGT GCT TTG AGT GAA GGG GGC TTT GCA AGT GCG CTG ACA TTT GGA...	pHDS58
A3/IIIC5	8.F3.4	...TGT GCT TTG AGT GAA GGG GGC TTT GCA AGT GCG CTG ACA TTT GGA...	pHDS58
A24/PEF8	8.F3.4	...TGT GCT TTG AGT GAG GGG GGC TTT GCA AGT GCG CTG ACA TTT GGA...	pHDS58
223/5	8.p71	...TGT GCT TTG AGT AAG ACA GGC TTT GCA AGT GCG CTG ACA TTT GGA...	pHDS58
223/27	8.p71	...TGT GCT TTG AGT GAC AAG GGC TTT GCA AGT GCG CTG ACA TTT GGA...	pHDS58
A24/PEF5	8.p71	...TGT GCT TTG AGT GTC GGA GGC TTT GCA AGT GCG CTG ACA TTT GGA...	pHDS58
223/14	8.F3.6	...TGT GCT CTG AGT CCT GGG GGC TTT GCA AGT GCG CTG ACA TTT GGA...	pHDS58
A3/C32b	8.F3.6	...TGT GCT CTG AGT GAA GGG GGC TTT GCA AGT GCG CTG ACA TTT GGA...	pHDS58
332/1K	3.A3/63	...TGT GCT GTG AGC GCG GGA GGC TTT GCA AGT GCG CTG ACA TTT GGA...	pHDS58
A3/63	3.A3/63	...TGT GCT GTG AGC GCG GGG GGC TTT GCA AGT GCG CTG ACA TTT GGA...	pHDS58
A3/IIC7	3.3	...TGT GCT GTG AGC GCG CGG GGC TTT GCA AGT GCG CTG ACA TTT GGA...	pHDS58
A3/74.1	3.A8	...TGT GCT CTG AGC ATG GGA GGC TTT GCA AGT GCG CTG ACA TTT GGA...	pHDS58
A3/H2R2	3.2	...TGT GCT GCG AGG CCC AGA GGC TTT GCA AGT GCG CTG ACA TTT GGA...	pHDS58
A3/C46b	3.C9	...TGT GCT GTG AGC GCG GGG GGC TTT GCA AGT GCG CTG ACA TTT GGA...	pHDS58
A2/25	4.BDFLII	...TGT GTT CTG GGT GAT CGG GGC TTT GCA AGT GCG CTG ACA TTT GGA...	pHDS58
332/2A	4.PJR25	...TGC GCT CTG AGT GAA AGA GGC TTT GCA AGT GCG CTG ACA TTT GGA...	pHDS58
A24/PEF1	5.MDA	...TGC GCA GTC AGT GCG GGA GGC TTT GCA AGT GCG CTG ACA TTT GGA...	pHDS58
A24/PEG2	5.MDA	...TGC GCA GTC AGT GGG GGC TTT GCA AGT GCG CTG ACA TTT GGA...	pHDS58
A24/PEF2	T2.5-5	...TGT GCA GCA AGC ATG GCA GGC TTT GCA AGT GCG CTG ACA TTT GGA...	pHDS58
A24/10.1	T2.5-5	...TGT GCA GCC TCG GGG GGC TTT GCA AGT GCG CTG ACA TTT GGA...	pHDS58
332/2G	T2.5-5	...TGT GCA GCA AGC GGG GGC TTT GCA AGT GCG CTG ACA TTT GGA...	pHDS58
A24/12.2	10.109S	...TGT GCT CCT GCG CAG ACA GGC TTT GCA AGT GCG CTG ACA TTT GGA...	pHDS58
A3/H2R5	10.FN1-18	...TGT GCT ATG GAA CGT GGG GGG GGC TTT GCA AGT GCG CTG ACA TTT GGA...	pHDS58
A3/72.2	13.2	...TGT GCT GTC ACG GGT TAC CAG AAC TTC TAT TTT GGG...	TA65
A24/PEF4	6.δ3	...TGT ATC CTG AGT AGA GGT TCA GCC TTA GGG AGG CTG CAT TTT GGA...	34S-281

B TCR β cDNAs

CTL clone	Vβ		Jβ
A3/C32b	10	...TGT GCC AGC AGC TTG GGA TCC GAC TAC ACC TTC GGC...	1.2
A3/IC1	10	...TGT GCC AGC AGC TTT GGC TCC GAC TAC ACC TTC GGC...	1.2
332/1K	10	...TGT GCC AGC AGC CAG GGG TCC GAC TAC ACC TTC GGC...	1.2
A3/IIC7	10	...TGT GCC AGC AGC TAC GGC TCC GAC TAC ACC TTC GGC...	1.2
332/2A	10	...TGT GCC AGC AGC TAC GGC TCC GAC TAC ACC TTC GGC...	1.2
A3/C46b	10	...TGT GCC AGC AGC TAT GGA CAC GAC TAC ACC TTC GGC...	1.2
A24/PEF1	10	...TGT GCC AGC AGC TTC GGC CCC GAC TAC ACC TTC GGC...	1.2
A24/10.1	10	...TGT GCC AGC AGC CTA GGG AAC ACC TTG TAC TTT GGT...	2.4
A3/63	10	...TGT GCC AGC AGC ACA GGA AAC ACC TTG TAC TTT GGT...	2.4
332/2G	10	...TGT GCC AGC AGC TTC GGA AAC ACC TTG TAC TTT GGT...	2.4
A3/74.1	10	...TGT GCC AGC AGC TTG GGA CAA GAA GTC TTC TTT GGT...	1.1
223/27	10	...TGT GCC AGC AGC TTT GGA CAG GGA GTC TTC TTT GGT...	1.1
A24/PEF2	10	...TGT GCC AGC AGC TTT GGA AAT ACG CTC TAT TTT GGA...	1.3
A3/H2R2	10	...TGT GCC AGC AGC TTT GGG GAA ACG CTG TAT TTT GGC...	2.3
A24/PEF8	10	...TGT GCC AGC AGC TCC GGA CAG ACG GAG TAC TTC GGT...	2.7
A24/PEF4	10	...TGT GCC AGC AGC CGG GAC AAC CAA GAC ACC CAG TAC TTT GGG...	2.5
A24/12.2	10	...TGT GCC AGC AGC CTC GGG ACA GGG AAC AAC GAA AGA TTA TTT TTC GGT...	1.4
A3/IIIC5	10	...TGT GCC AGC AGC TAT GGG ACA GGG ACC AAC GAA AGA TTA TTT TTC GGT...	1.4
A2/25	10	...TGT GCC AGC AGC TTC GGG ACA GGG AAC AAC GAA AGA TTA TTT TTC GGT...	1.4
223/5	10	...TGT GCC AGC AGC TCC GGG ACA GGG GAA AGG GAA AGA TTA TTT TTC GGT...	1.4
223/14	10	...TGT GCC AGC AGC TTC GGG ACA GGG GAA AAC GAA AGA TTA TTT TTC GGT...	1.4
A3/H2R5	10	...TGT GCC AGC AGC GTC GGG ACA GGG CCC AAC GAA AGA TTA TTT TTC GGT...	1.4
A24/PEF5	10	...TGT GCC AGC AGC ACC GGG ACA GGG ACC AAC GAA AGA TTA TTT TTC GGT...	1.4
A3/72.2	1	...TGT GCC AGC AGC CAA GCT GGG TAT GAA CAG TAC TTC GGT...	2.7
A3/C80b	8.3	...TGT GCC AGC AGT ACC GGG ACA GGG ACC AAC GAA AGA TTA TTT TTC GGT...	1.4
A24/PEG2	15	...TGT GGT GCT GCC GAG GGT GCT GAG CAG TTC TTC GGA...	2.1

Figure 3. TCR α and β cDNA junctional nucleotide sequences of A24-selected CTL clones. (*A*) TCR α junctional sequences. The nomenclature for the Vα subfamilies follows that of references 3, 29, and 30. Here, the Vα subfamily is separated from the Vα gene segment by a period. The Vα gene segments are named according to their original description: from references 31 (Vα5.MDA, Vα8.p71, Vα10.FN1–18), 32 (Vα8.F3.4, Vα8.F3.6), 25 (Vα3.2, Vα3.3), 33 (Vα4.BDFLII), 34 (Vα4.PJR-25), 35 (Vα10.109S), 36 (Vα13.2), 37 (Vα3.C9), 14 (Vα3.A8), 18 (VαT2.5–5), and 38 (Vα6.δ3), and this report (Vα3.A3/63). Due to partial sequencing, the Vα segment identification is only putative. The partial sequence of the Vα3.A3/63 gene segment is available upon request. All other segments but one are 100% identical at the nucleotide level to the published ones in the region sequenced, namely downstream of the Vα primer used for PCR and sequence. The Vα3.C9 gene segment partial sequence in CTL clone A3/C46b is identical to that of CTL clone Cw3/1F11 (14), differs by a single base substitution from the original sequence, and is available upon request. The VαT2.5–5 partial sequence is identical to the one previously referred to as Vα5T.J3 (15). The JαpHDS58 gene segment is from references 39 and 40, the JαTA65 is from reference 29, and the Jα34S-281 is from reference 41. (*B*) TCR β cDNA junctional sequences. The nomenclature for the Vβ gene segments follows that of references 3 and 42–45. The Vβ1, Vβ8.3, Vβ10, and Vβ15 sequences were originally reported in references 46, 42, 47, and 43, respectively. The Jβ gene segments sequences are from references 48 and 49, except Jβ1.4, which is from reference 15. Unambiguously assigned Dβ gene segments (50, 51) are underlined. These sequences are available from EMBL/GenBank/DDBJ under accession numbers X70709–X70763.

TCRs. As a control, the fragments amplified from LNs of nonimmunized mice were not readable at all, reflecting the extensive diversity of Vβ10-Jβ1.2 junctions among unselected lymphocyte populations.

Moreover, the apparent distinction between the TCR repertoires from CTL clones selected by HLA-Cw3 and HLA-A24, in terms of length of the Vβ10-Jβ1.4 CDR3, was also evident in vivo, since PELs from Cw3- or A24-immunized mice displayed Vβ10-Jβ1.4 loops of mutually exclusive lengths of 6 and 10 amino acids, respectively (Fig. 6). Thus, a distinctive feature of the TCR β junctional region repertoire, obtained from the comparison of a series of Cw3- and A24-selected CTL clones isolated and grown in vitro, was found to be relevant in vivo.

These findings support the conclusions drawn from the analysis of CTL clones isolated and grown in vitro, and indicate that the TCR β junctional region may be critical in recognition of position 173 of the HLA peptides in vivo.

Discussion

For the CTL clones selected either by Cw3 or A24 that do crossrecognize the other HLA allele, interaction with position 173 does not appear to be critical to the overall recog-

CTL clone	TCR β					TCR α					REACTIVITY	
	Vβ	FW	CDR3	FW	Jβ	Vα	FW	CDR3	FW	Jα	CW3	A24
GROUP I												
			99					94				
A3/C32b	10	CAS	S L G S D Y	TFG	1.2	8.F3.6	CAL	S E G G F A S A L	TFG	pHDS58	NT	
A3/IC1	10	CAS	S F G S D Y	TFG	1.2	8.F3.4	CAL	S E G G F A S A L	TFG	pHDS58	NT	
332/1K	10	CAS	S Q G S D Y	TFG	1.2	3.A3/63	CAV	S A G G F A S A L	TFG	pHDS58		
A3/IIC7	10	CAS	S Y G S D Y	TFG	1.2	3.3	CAV	S A R G F A S A L	TFG	pHDS58	NT	
332/2A	10	CAS	S Y G S D Y	TFG	1.2	4.PJR25	CAL	S E R G F A S A L	TFG	pHDS58		
A3/C46b	10	CAS	S Y G H D Y	TFG	1.2	3.C9	CAV	S A G G F A S A L	TFG	pHDS58	NT	
A24/PEF1	10	CAS	S F G P D Y	TFG	1.2	5.MDA	CAS	S A G G F A S A L	TFG	pHDS58		
A3/H2R2	10	CAS	S F G E T L	YFG	2.3	3.2	CAA	R P R G F A S A L	TFG	pHDS58	NT	
A3/74.1	10	CAS	S L G Q E V	FFG	1.1	3.A8	CAL	S M G G F A S A L	TFG	pHDS58	▨	
223/27	10	CAS	S F G Q G V	FFG	1.1	8.p71	CAL	S D K G F A S A L	TFG	pHDS58	■	
A24/PEF8	10	CAS	S S G Q T E	YFG	2.7	8.F3.4	CAL	S E G G F A S A L	TFG	pHDS58		
GROUP II												
A3/IIIC5	10	CAS	S Y G T G T N E R L	FFG	1.4	8.F3.4	CAL	S E G G F A S A L	TFG	pHDS58	NT	
A2/25	10	CAS	S F G T G N N E R L	FFG	1.4	4.BDFLII	CVL	G D R G F A S A L	TFG	pHDS58	■	
223/5	10	CAS	S S G T G E R E R L	FFG	1.4	8.p71	CAL	S K T G F A S A L	TFG	pHDS58	■	
223/14	10	CAS	S F G T G E N E R L	FFG	1.4	8.F3.6	CAL	S P G G F A S A L	TFG	pHDS58	■	
A24/PEF5	10	CAS	S T G T G T N E R L	FFG	1.4	8.p71	CAL	S V G G F A S A L	TFG	pHDS58	NT	
A3/63	10	CAS	S T G N T L	YFG	2.4	3.A3/63	CAV	S A G G F A S A L	TFG	pHDS58	■	
GROUP III												
A24/10.1	10	CAS	S L G N T L	YFG	2.4	T2.5-5	CAA	S G G F A S A L	TFG	pHDS58	■	
332/2G	10	CAS	S F G N T L	YFG	2.4	T2.5-5	CAA	S G G F A S A L	TFG	pHDS58	■	
A24/PEF2	10	CAS	S F G N T L	YFG	1.3	T2.5-5	CAA	S M A G F A S A L	TFG	pHDS58	■	
A24/PEF4	10	CAS	S R D N Q D T Q	YFG	2.5	6.δ3	CIL	S R G S A L G R L	HFG	34s-281	■	
A24/12.2	10	CAS	S L G T G N N E R L	FFG	1.4	10.109S	CAP	A Q T G F A S A L	TFG	pHDS58	▨	
A3/H2R5	10	CAS	S V G T G P N E R L	FFG	1.4	10.FN1-18	CAM	E R G G G F A S A L	TFG	pHDS58	NT	
A3/C80b	8.3	CAS	S T G T G T N E R L	FFG	1.4	8.F3.4	CAL	S D G G F A S A L	TFG	pHDS58	NT	
A24/PEG2	15	CAS	A E G A E Q	FFG	2.1	5.MDA	CAV	S G G F A S A L	TFG	pHDS58	■	
A3/72.2	1	CAS	S Q A G Y E Q	YFG	2.7	13.2	CAV	T G Y Q N F	YFG	TA65	■	

Figure 4. TCR α and β chains of A24-selected CTL clones and their crossrecognition of Cw3. The 26 CTL clones are listed on the vertical axis. For each clone, the in-frame TCR β transcript encoding the key residues at the VDJ junction (6) was considered to encode the functional TCR β chain. For CTL clones A24/PEF1, 2, 4, 5, and 8, a FACS® staining with the anti-Vβ10 mAb B21.5 (52) was performed to confirm the β transcript assignment. The deduced amino acid sequences of the junctional and hypervariable regions, putatively CDR3-like, are reported (in single-letter amino acid code) according to reference 6. The presumed Ig-like loops, designated CDR3 for convenience, are putatively CDR3-like, are reported (in single-letter amino acid code) according to reference 6. The presumed Ig-like loops, designated CDR3 for convenience, are putatively supported by two framework branches (FW), which are also reported here. The key Cys residue is at positions 90 and 92 in the α and β chains, respectively. The Vα, Vβ, Jα, and Jβ segments are also reported (see Fig. 3 for references). Regions of the TCR α or β chains expressed by the A24-selected CTL clones that differ from those expressed by Cw3-selected CTL clones were boxed. The α transcript can be unambiguously assessed to encode the functional α chain, i.e., paired with the β chain to form a heterodimer specific for the HLA-A24 peptide–H-2K^d complex, only when a second, out-of-frame transcript was also detected (15, 53). An out-of-frame α transcript was found in CTL clones A24/12.2, PEF4, A3/H2R2, H2R5, 72.2, and 332/2A, and a second α transcript, in-frame at the V-J junction, was found in CTL clones A24/10.1 and 322/1K. However, for the latter two and the remaining CTL clones, the α transcripts reported here are likely to encode the functional α chains because of their structural homology with the unambiguously assigned ones. All CTL clones recognize the HLA-A24 170–179 antigenic site (□). Level of crossrecognition of the Cw3 170–179 antigenic site is indicated: (□) recognition of Cw3 as well as that of A24; (▨) intermediate recognition; or (■) no recognition. Clones that were not tested for recognition of Cw3 are indicated (NT). Clones A3/74.1 and 72.2 were tested for recognition of P815-Cw3 and A3 transfectant cells and for recognition of Cw3 and A24 170–182 peptides, and clone A3/72.2 was further tested for recognition of Cw3 and A24 170–179 peptides. Clones A24/10.1 and A24/12.2 were tested for recognition of P815-A24 and Cw3 transfectant cells, and for recognition of Cw3 and A24 170–182 peptides, and clone A24/10.1 was further tested for recognition of Cw3 and A24 170–179 peptides. Clones A24/PEF1, PEF2, PEF4, PEF8, and PEG2 were tested for recognition of Cw3 and A24 170–179 peptides. Clones 223/5, 223/14, 223/27, 332/1K, 332/2A, 332/2G, A2/25, and A3/63 were tested for recognition of P815-Cw3 and A24 transfectant cells and for recognition of Cw3 and A24 170–179 peptides. E/T ratios or peptide concentrations were titrated to evaluate the relative recognition of transfectants or peptides, respectively.

nition of the peptide/MHC complex. Therefore, regions of the TCRs expressed by these CTLs, if any, contacting this residue cannot be deduced from this structure-function analysis. In contrast, the comparison of the noncrossreactive CTL clones, strictly specific for either Cw3 or A24, provides evidence that the TCR β junctional regions expressed by these CTL interact with position 173 in the amino-terminal part of the HLA peptides.

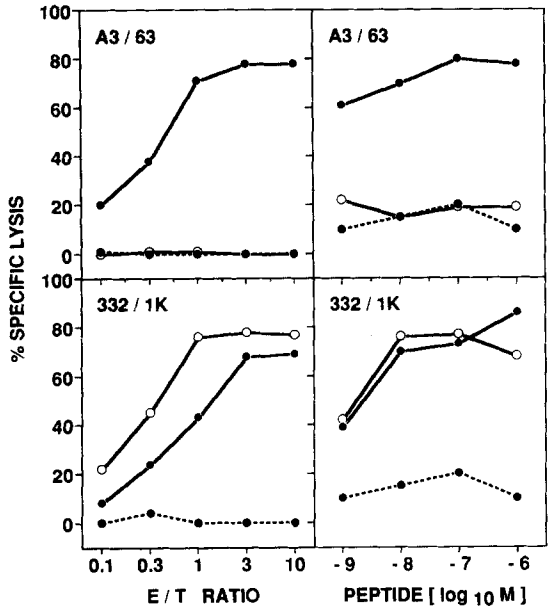

Figure 5. Crossrecognition of Cw3 by a set of A24-selected CTL clones. Two A24-selected CTL clones, 332/1K and A3/63, were tested for recognition either of P815 cells transfected with the HLA-Cw3 (O) or A24 (●) genes (*left*); or of P815 (H-2^d) target cells pulsed with HLA-Cw3 (O) or A24 (●) 170–179 peptides (*right*). Lysis of control P815 cells without peptide is also shown (*dashed lines*).

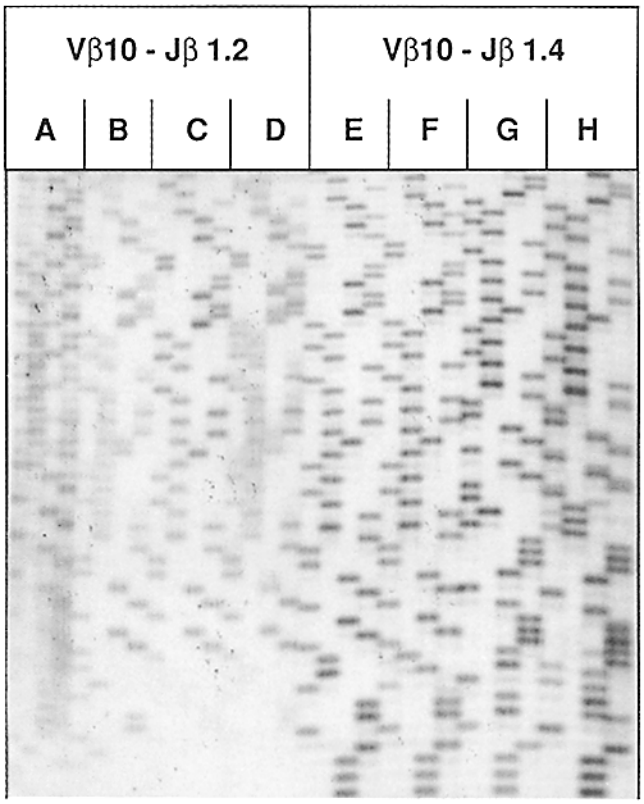

Figure 6. Direct sequencing of TCR junctional regions expressed in vivo by PELs from immune mice. The PELs originating from DBA/2 mice immunized with P815-Cw3 or P815-A24 transfectant cells (two individual mice for each antigen) were purified by passage over nylon wool. The RNA was extracted from each of the four PEL samples, from the CTL clones Cw3/1.1, Cas7, and A24/12.2, and from normal DBA/2 lymph nodes. After cDNA synthesis, the Vβ10-Jβ1.2 junctional regions were amplified by PCR using a combination of Vβ10 and Jβ1.2 primers. The noncoding strand of each double-stranded PCR product was directly sequenced using the antisense Jβ1.2 primer. The four samples (*A*, normal lymph nodes; *B*, CTL clone Cw3/1.1; *C*, anti-A24 PELs from one individual mouse; *D*, anti-Cw3 PELs from one individual mouse) were loaded on the same sequencing gel in the GATC order. The sequences obtained from the other two animals gave similar results (not shown). In a second experiment, the Vβ10-Jβ1.4 junctional regions were amplified by PCR using a pair of Vβ10-Jβ1.4 primers, and were sequenced with the antisense Jβ1.4 primer. The four samples (*E*, CTL clone Cw3/Cas7; *F*, anti-Cw3 PELs from one individual mouse; *G*, CTL clone A24/12.2; *H*, anti-A24 PELs from one individual mouse) were loaded in the GATC order on the same sequencing gel as the Vβ10-Jβ1.2 sequences. The sequences obtained from the other two animals gave similar results (not shown). The sequenced noncoding strand of each PCR product can be read from bottom to top (GATC from left to right).

Three groups have independently proposed a model in which the α and β CDR3 loops of the TCR would primarily interact with the antigenic peptide, whereas the CDR1 and 2 would contact the MHC restriction element (6, 20, 21). These models were essentially based on the much higher variability of the CDR3 and antigenic peptides, when compared with the CDR1, 2, and MHC molecules, respectively.

Experimental evidence supporting this model, and in particular that TCR CDR3 loops were critical for recognition of the antigenic peptide, has been provided in various class II MHC-restricted systems. Conclusions were mainly based on the differences of peptide fine specificities displayed by T cells bearing TCRs, either natural variants (22–25) or genetically engineered (26, 27), that differed at a single position in either of the CDR3 loops.

Of the four studies in which the effect of a single substitution in the antigenic peptide was analyzed, two suggest that both the CDR3 α and β are critical for recognition of the same residue in the carboxy terminus either of the λ repressor (23) or of the cytochrome *c* (27) peptides. Two others suggest that the CDR3 α interacts with a residue in the carboxy-terminal part or in the amino-terminal part of the myoglobin (22) or hemagglutinin (24) peptides, respectively.

The orientations may indeed differ from case to case. Alternatively, elements other than the TCR sequences, which vary from cell to cell, may also affect recognition patterns, as seems to be the case in the present study among Cw3-selected CTL clones tested for crossrecognition of A24. These include level of TCR, level of adhesion molecules, sensitivity of activation pathways, etc. In addition, correlations between TCR primary structure and fine specificity may not necessarily reflect only the direct interactions of the structures involved, but also differences in affinity provided by interactions at other places.

To settle this issue, an elegant approach, which consists of analyzing the TCR repertoire in single chain TCR transgenic mice immunized with variant peptides substituted at a single position, has been reported recently in the class II MHC-restricted response to cytochrome *c* (28). The results indicate that both CDR3 α and β are critical for peptide

recognition, and that the CDR3 β would interact with the carboxy-terminal part of the peptide, whereas the CDR3 α would interact with the amino-terminal part of the peptide.

We have adopted a similar approach to the class I MHC-restricted response to HLA-Cw3 with two modifications. First, we have immunized normal mice, which were free to adapt either of the TCR chains in response to the single amino acid substitution. Second, we have analyzed the TCR repertoire used in vivo by harvesting PELs obtained from immune animals in order to confirm the findings obtained on T cell clones isolated and grown in vitro.

Altogether, our findings provide evidence that the TCR β junctional regions interact with the amino-terminal part of the HLA peptides presented by a class I MHC molecule. Notably, this orientation differs from that found in the class II MHC-restricted response to cytochrome *c* (28). Further experiments are required to determine whether the orientation of the TCRs on the surface of MHC/peptide complexes varies according to the class of the MHC or to the nature of the peptide.

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