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-2Kd for recognition by murine cytolytic T lymphocytes (CTLs). As a first approach to understand the way T cell receptors (TCRs) interact 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 Iggs (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-2Kd, 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-2Kd 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-2Kd-restricted CTLs (13).

We recently showed that the TCRs carried by H-2Kd-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 VB segment (VB1), a single Jα segment (JαHDSS), 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-
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\(^2\) binding motif (underlined) \((2, 11, 12)\) and bind equally well to K\(^2\) (not shown).

The usage of the only two variable segments of the TCR repertoire selected by Cw3, namely J3 and Vcr, was not evenly distributed between crossreactive and noncrossreactive clones. As illustrated in Fig. 2, only three dones appear to recognize the latter category, the patterns were in fact heterogeneous, whereas none of the 11 noncrossreactive ones did. The frequency of negatively charged residues at positions 94 in the CDR3 \(\alpha\), mostly Vo\(\alpha\)4 and Vo\(\alpha\)8 encoded, was higher among crossreactive (16/19) than noncrossreactive (6/11) CTL clones.

**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 J\(\beta\)1.2 and J\(\beta\)1.4 primers will be reported elsewhere (Pannetier et al., manuscript in preparation). Sequences of the newly designed primers specific for the V\(\alpha\)7 T2.5-5 (18) and V\(\alpha\)B6.2.16 subfamilies (19) are CAGACACACAGCCAAAGAC and GAGACACGGTGGTTAAAGGC, 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\(^4\)-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 J3 and V\(\alpha\), was not evenly distributed between crossreactive and noncrossreactive clones.

The J\(\beta\)1.4 and J\(\beta\)2.3 segments were more frequent among noncrossreactive (6/11) than crossreactive (3/19) clones, whereas the reverse trend was found for J\(\beta\)1.2. The Vo\(\alpha\)3 subfamily was more frequent among noncrossreactive (5/11) than crossreactive (1/19) clones, whereas the reverse trend was found for Vo\(\alpha\)4 and Vo\(\alpha\)8.

The amino acids occurring at two positions of the CDR3 \(\alpha\) also showed some bias with respect to crossreactivity pattern. At position 95, many (11/19) crossreactive clones expressed a non-V\(\alpha\)-, 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 \(\alpha\), mostly Vo\(\alpha\)4 and Vo\(\alpha\)8 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 \(\alpha\) or \(\beta\) 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 \(\beta\) chains, but different reactivities, were found. Clones Cw3/4A3, HLA1G6, PEA1, and PEA34 crossreact with A24, unlike PEA13, although they share the same TCR \(\beta\) sequence. Notably, the former clones are all Vo\(\alpha\)8 and their CDR3 \(\alpha\) contain a pair of complementary charges, while the latter clone is Vo\(\alpha\)3 and its CDR \(\alpha\) contains only an acid charge. Similarly, clones Cw3/PEA30 and HLA1C8 share the same TCR \(\beta\) sequence and are both Vo\(\alpha\)4, but the former does crossreact and its CDR3\(\alpha\) contains a pair of opposite charges, whereas the latter does not crossreact and its CDR3\(\alpha\) only contains an acid residue. Finally, whereas the clone Cw3/C37 is Vo\(\alpha\)8, with a CDR3 \(\alpha\) containing two complementary charges, the clone HLA2D3 is Vo\(\alpha\)4, its CDR3 \(\alpha\) contains no charged residue, and only the former clone crossreacts although they both display the same TCR \(\beta\) sequence.

Conversely, CTL clones sharing an identical TCR \(\alpha\) chain (although, rigorously, divergence in the V\(\alpha\) 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 \(\alpha\) chain, but only the latter does not crossreact with A24. Its TCR \(\beta\) differs from that of the two other clones in J\(\beta\) segment usage (J\(\beta\)2.3 instead of J\(\beta\)1.2 or J\(\beta\)2.7).

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 \(\alpha\)/\(\beta\) 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.

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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 amino acids occurring at two positions of the CDR3 \(\alpha\) also showed some bias with respect to crossreactivity pattern. At position 95, many (11/19) crossreactive clones expressed a non-V\(\alpha\)-, 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 \(\alpha\), mostly Vo\(\alpha\)4 and Vo\(\alpha\)8 encoded, was higher among crossreactive (16/19) than noncrossreactive (6/11) CTL clones.

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1. **Abbreviation used in this paper:** PEL, peritoneal exudate lymphocyte.
Table 2. Origin of H-2K<sup>e</sup>-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/IC7 | This report* |
| 5 DBA/2 | P815-A3/hβ2m | P815-A3/hβ2m | A3/H2R2, H2R5 | This report* |
| 6 DBA/2 | P815-A3/hβ2m | P815-A3/hβ2m | A3/C32b, C46b, C80b | This report* |
| 7 DBA/2 | P815-A2 | P815-A2 | A2/25 | 13 |
| 8 DBA/2 | P815-A2 | P815-A2 | A3/63 | 13 |
| 9 DBA/2 | P815-A2 | P815-A2 | 223/5, 14, 27 | 13 |
| 10 DBA/2 | P815-A2 | P815-A2 | 332/1K, 2A, 2G | 13 |
| 11 DBA/2 | P815-A2 | P815-A2 | A24/PEF1, 2, 4, 5, 8 | This report* |
| 12 DBA/2 | P815-A2 | P815-A2 | 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<sup>e</sup> 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<sup>e</sup>.

* 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 derived by limiting dilution of CD8<sup>+</sup> 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<sup>e</sup>-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 V<sub>β</sub>10 and J<sub>α</sub>PD2S58 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 V<sub>β</sub>10 and J<sub>α</sub>PD2S58 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.

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 V<sub>β</sub>10-J<sub>β</sub>1.4. In contrast, all V<sub>β</sub>10-J<sub>β</sub>1.4 CDR3 loops in the anti-Cw3 TCR repertoire were of six amino acids. In addition, one clone uses the J<sub>β</sub>2.4 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 V<sub>α</sub> subfamily.

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 functional region, again either in the usage of different J<sub>β</sub> segments (J<sub>β</sub>2.4, 1.3, 2.5, 2.1), or of different CDR3 lengths (V<sub>β</sub>10-J<sub>β</sub>1.4 loops of 10 amino acids). Additional changes can affect every part of the heterodimers: V<sub>β</sub> segment usage, CDR3 β length, lack of glycine at position 97 in the CDR3 β, V<sub>α</sub> subfamilies usage, CDR3 α length, and J<sub>α</sub> segment usage.
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 Vo3 subfamily usage and charged amino acid composition at positions 94 and 95 of the CDR3 α.

The frequency of Vo3 among A24-selected clones (6/26) is similar to that of Cw3-selected clones (6/30), although of the latter Vo3 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 of the latter Voe3 clones, most did not crossrecognize A24.

These results suggest that the Vo 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 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.

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

| CTL clone | TCR β | TCR α | REACTIVITY |
|-----------|-------|-------|------------|
| CW3/1.1 | FW CDR3 | FW Jβ | Vo CDR3 | FW Ju | CW3 | A24 |
| CW3/Cws/20 | FW CDR3 | FW Jβ | Vo CDR3 | FW Ju | CW3 | A24 |
| CW3/Cw3/21 | FW CDR3 | FW Jβ | Vo CDR3 | FW Ju | CW3 | A24 |
| CW3/Cw3/22 | FW CDR3 | FW Jβ | Vo CDR3 | FW Ju | CW3 | A24 |
| CW3/Cw3/23 | FW CDR3 | FW Jβ | Vo CDR3 | FW Ju | CW3 | A24 |
| CW3/Cw3/24 | FW CDR3 | FW Jβ | Vo CDR3 | FW Ju | CW3 | A24 |
| CW3/Cw3/25 | FW CDR3 | FW Jβ | Vo CDR3 | FW Ju | CW3 | A24 |
| CW3/Cw3/26 | FW CDR3 | FW Jβ | Vo CDR3 | FW Ju | CW3 | A24 |
| CW3/Cw3/27 | FW CDR3 | FW Jβ | Vo CDR3 | FW Ju | CW3 | A24 |
| CW3/Cw3/28 | FW CDR3 | FW Jβ | Vo CDR3 | FW Ju | CW3 | A24 |
| CW3/Cw3/29 | FW CDR3 | FW Jβ | Vo CDR3 | FW Ju | CW3 | A24 |
| CW3/Cw3/30 | FW CDR3 | FW Jβ | Vo CDR3 | FW Ju | CW3 | A24 |
| CW3/Cw3/31 | FW CDR3 | FW Jβ | Vo CDR3 | FW Ju | CW3 | A24 |
| CW3/Cw3/32 | FW CDR3 | FW Jβ | Vo CDR3 | FW Ju | CW3 | A24 |
| CW3/Cw3/33 | FW CDR3 | FW Jβ | Vo CDR3 | FW Ju | CW3 | A24 |
| CW3/Cw3/34 | FW CDR3 | FW Jβ | Vo CDR3 | FW Ju | CW3 | A24 |

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 Vα, Vβ, and β 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 putatively derived from another region, possibly contributing to the crossreaction patterns observed among Cw3-selected clones.
frequency was lower among the clones that did not crossrecognize A24 than among those that did.

These results in turn suggest that the TCR \(\beta\) junctional region is critical for recognition of position 173. Nevertheless, the differences observed in the TCR repertoires selected by Cw3 and A24 may result 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 crossrecognition 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 J\(\beta1.1\) segment and has therefore been classified in group I. However, this segment was found in only 2 of 57 Cw3-selected CTL clones, which may in part explain the somewhat lower crossactivity 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 J\(\beta1.1\) segment, but also does not express any acid residue in the CDR3 due to extensive trimming of the J\(\beta1.1\) 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 \(\alpha\) chain, and the comparison of their crossreactivity supports our previous conclusions. These two clones bear identical TCR \(\alpha\) chains, but whereas 332/1K crossrecognizes 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 \(\beta\) 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 \(\beta\) 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 V\(\beta10\) among CD8 cells was significantly increased, indicating that the V\(\beta10\) dominance observed on CTL clones also occurred in vivo (not shown). However, among A24-selected PELs, the higher variation of the V\(\beta10\) 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 V\(\beta\) segments may occasionally be selected by A24, as was the V\(\beta15\) CTL clone PEG2, derived from one mouse in which no V\(\beta10\) increase was observed.

To analyze the TCR response in more detail, a first cDNA PCR with a V\(\beta10\) and a J\(\beta1.2\) pair of primers was performed on the Cw3- and A24-specific PELs, and the product was directly sequenced with the J\(\beta1.2\) 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 V\(\beta10\)-J\(\beta1.2\)
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-
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.

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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 and 2 would contact the MHC restriction element (6, 20, 21). These models were essentially based on the much higher variability and would primarily interact with the antigenic peptide, whereas 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, has been reported recently in the class II MHC-restricted response to cytochrome c (28). The results of analyzing the TCR repertoire in single chain TCR 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).

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.

Overall, 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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