H-2-restricted Cytolytic T Lymphocytes Specific for HLA Display T Cell Receptors of Limited Diversity

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Summary
We previously showed that H-2Kd-restricted cytotoxic T lymphocyte (CTL) clones specific for a single nonapeptide derived from the Plasmodium berghei circumsporozoite (PbCS) protein displayed T cell receptors (TCRs) of highly diverse primary structure. We have now analyzed the TCR repertoire of CTLs that recognize a peptide derived from the human class I major histocompatibility complex (MHC) molecule HLA-Cw3 in association with the same murine class I MHC molecule H-2Kd. We first sequenced the TCR γ and β genes of the CTL clone Cw3/1.1 and, based on this genomic analysis, the TCR γ and β cDNA junctional regions of 23 independent H-2Kd-restricted CTL clones specific for HLA-Cw3. The results show that the TCR chains display very limited heterogeneity, both in terms of Vγ, Joγ, Vβ, and Jβ segments, and in terms of length and sequence of the CDR3 γ and β loops. The TCR repertoire used in vivo was then analyzed by harvesting CTL populations from the peritoneal cavity of immune mice. The peritoneal exudate lymphocytes (PELs) displayed HLA-Cw3-specific cytolytic activity in the absence of any stimulation in vitro. Remarkably, most of these freshly isolated PELs expressed TCRs that shared the same structural features as those from HLA-Cw3-reactive CTL clones. Thus, our results show that a peptide from HLA-Cw3 presented by H-2Kd selects CTLs that bear TCRs of very limited diversity in vivo. When taken together with the high diversity of the TCRs specific for the PbCS peptide, these findings suggest that natural tolerance to self peptides presented by class I MHC molecules may substantially reduce the size of the TCR repertoire of CTLs specific for antigenic peptides homologous to self.

Class I MHC molecules carry peptides derived from intracellular antigens to the cell surface (1), where the molecular complex is recognized by CTLs (2). The specificity of CTL recognition is assumed by the αβ TCR (3). Both α and β chains contain a constant (C) and a variable (V) extracellular domain. The V domain diversity results from the somatic recombination of a number of V, (D), and J gene segments, imprecise joining, and addition of template-independent N nucleotides (4). This extensive receptor diversity allows peripheral T cells to respond to a wide variety of antigenic challenges.

We previously showed that the TCRs carried by a large series of H-2Kd-restricted CTL clones specific for a single Plasmodium berghei circumsporozoite (PbCS)1 nonapeptide were highly diverse, both in terms of Vα, Jα, and Jβ segments, and in terms of amino acid composition of the junctional regions, despite a VB segment dominance (5).

We have now analyzed the TCR repertoire of H-2Kd-restricted CTLs specific for P815 (H-2b) cells transfected with the HLA-Cw3 gene (6). The optimal synthetic peptide recognized by these CTLs corresponds to the 10-mer HLA-Cw3 170-179 (7, 8), and shows significant homology with self peptides derived from the same region of the class I H-2d molecules.

We found that the TCRs carried by H-2Kd-restricted CTLs specific for HLA-Cw3 display very limited heterogeneity not only in vitro, but also in vivo. Taken together with the high diversity of the TCRs specific for the PbCS peptide, our findings suggest that tolerance to self peptides may reduce the available TCR repertoire of CTLs specific for antigenic peptides homologous to self.

1 Abbreviations used in this paper: PbCS, Plasmodium berghei circumsporozoite; PEL, peritoneal exudate lymphocyte.
**Materials and Methods**

**CTL Clones.** The origin of the HLA-Cw3-reactive CTL clones used in this study is summarized in Table 1. The cells were maintained in culture as described in the references.

**Peritoneal Exudate Lymphocytes (PELs).** DBA/2 (H-2b) mice were injected intraperitoneally with 2.5 × 10⁸ irradiated P815 cells transfected with the HLA-Cw3 gene (6). A second injection with 10⁸ cells was performed 5 wk later. Control C57BL/6 (H-2b) mice were injected with the same number of untransfected P815 cells. 6 d after the second injection, cells were harvested from the peritoneal cavity and PELs were purified by passage over a column of nylon wool (9). Aliquots of the recovered PELs were directly tested for specific cytolytic activity (6) or were stained for analysis by flow cytometry.

**Cell Surface Labeling with mAbs.** Staining was performed with B21.5 mAb (anti-Vβ10) supernatant (10) revealed by a goat anti-rat Ig FITC conjugate (Caltag Laboratories, San Francisco, CA), followed by biotinylated CD8 mAb 53-6.7 revealed by a tricolor avidin conjugate (Caltag Laboratories). The samples were passed on a FACSscan® flow cytometer (Becton Dickinson & Co., Sunnyvale, CA). Dead cells and debris were excluded by live gating on forward scatter (FSC) and SSC. Gated CD8+ cells were then analyzed for expression of Vβ10.

**Genomic Clones and Southern Blot Analyses.** The Cw3/1.1 T cell genomic library was constructed from Sau3A partially digested DNA using the λ-bacteriophage vector EMBL 3 (11). Screening and analysis of recombinant clones, as well as Southern blot analyses, were performed as previously described (12, 13).

**cDNA-PCR and Direct Sequencing.** RNA extraction, cDNA synthesis, and PCR with Vα, Vβ, Co, and Cβ primers were carried out on CTL clones or freshly isolated PELs as previously described (5). Direct sequencing of the double-stranded linear DNA product with Sequenase (US Biochemical, Cleveland, OH) and α-35S-dATP was carried out as originally described (14) with minor modifications (15).

**Results**

To analyze the TCR repertoire of H-2Kd-restricted CTLs specific for HLA-Cw3 within region 170–179, we first determined the sequence of the TCR α and β genes displayed by the CTL clone Cw3/1.1. Based on these results, we sequenced the TCR α and β cDNA junctional regions of 23 independent CTL clones of similar specificity. Finally, we analyzed the TCRs expressed by PELs freshly isolated from immunized mice.

**TCR α and β Gene Rearrangements in CTL Clone Cw3/1.1.** Genomic clones corresponding to the β gene rearrangements observed by Southern blot analyses were isolated from the Cw3/1.1 library, using probes Jβ1 and Jβ2 (13), and sequenced. One productive rearrangement involved the Vβ10 (4, 16) and Jβ1.2 gene segments (17) (Fig. 1) and resulted in 1.3-kb RNA transcripts (data not shown). The Dβ segment usage could not be unambiguously ascertained. The second VDJ rearrangement, out of the proper translational reading frame, involved the Vβ3, Dβ1, and Jβ2.1 gene segments (data not shown).

The structure of the TCR α gene rearrangements was analyzed by Southern blots using a panel of 17 single-copy probes encompassing the totality of the Jα gene segments and most of the Dβ-δβ-Cδ cluster (12). Genomic clones, hybridizing to probes 10 and 11, were isolated, characterized by restriction map analysis (Fig. 2), and sequenced (Fig. 3). The productive rearrangement, Cw3/1.1A, involved a member of the
Vo8 subfamily, whose coding region was 100% identical at the nucleotide level to the BALB/c Vo8.71 gene segment (18), and the JopHDS58 gene segment (19, 20). It resulted in 1.5-kb RNA transcripts (data not shown). Rearrangement Cw3/1.1 B, out of proper translational frame, involved a member of the Va5 subfamily with a coding region 100% identical at the nucleotide level to the VoMDA gene segment (18), referred to as Vo 5.3 gene segment (4, 18, 21), and the JαK1 segment (5), which we have positioned within the Jα cluster (Fig. 2).

**TCR β cDNA Junctional Regions of HLA-Cw3–specific CTL Clones.** A series of 45 H-2Kd-restricted CTL clones specific for HLA-Cw3 was submitted to cDNA-PCR with a sense Vβ10 primer in conjunction with an antisense CB primer. All clones were found to be positive, and the sequencing of the PCR products allowed the unambiguous identification of at least 23 independent CTL clones, based on differences either of TCR β gene sequence or of origin of animal (Fig. 4 and Table 1).

The 23 TCR β chains displayed remarkably conserved features (Fig. 5). All chains expressed the Vβ10 segment. Out of 12 possible Jβ segments, only 5 Jβ segments were found. The Jβ1.2 segment was expressed by a majority of the clones (12/23) and three segments, namely Jβ1.2, Jβ2.3, and Jβ1.4, constituted nearly all chains (20/23). All 23 TCR β chain junctional regions displayed the same CDR3 length, as defined by Chothia et al., (22), of six amino acids. A glycine residue, non V, non J-encoded, was strictly conserved in all chains at position 97. Subgroups of CTL clones bearing identical TCR β chain amino acid sequences were found. In addition, many β chains differed from others at only a single position in the CDR3 loop, often by a conservative substitution.

**TCR α cDNA Junctional Regions of HLA-Cw3–specific CTL Clones.** The 23 independent CTL clones were first submitted to cDNA-PCR with a sense Va8 primer in conjunction with an antisense Caα primer. Eight clones expressed a Va8 transcript and the remaining 15 clones were tested sequentially with primers corresponding to most Va8 subfamilies.

The sequences of the PCR products (Fig. 4) revealed closely conserved features among the TCR α chains (Fig. 6). Out of at least 22 possible Va subfamilies, only five were found and three of these, namely Va8, Va8, and Va8, constituted the vast majority of the repertoire (20/23). All chains bore the JαpHDS58 segment, out of around 50 possible Jα segments. Furthermore, a single length of nine amino acids was found for the CDR3 loop. A high proportion of aspartic or glutamic acid at position 94 (17/23) and of glycine or arginine residues at position 95 (16/23), non J-encoded, were found for the CDK3 loop. A high proportion of aspartic or glutamic acid at position 94 (17/23) and of glycine or arginine residues at position 95 (16/23), non J-encoded, were found for the CDK3 loop.
CTC analysis of freshly isolated PELs specific for HLA-Cw3. It has been shown previously that mice injected intraperitoneally with allogeneic irradiated tumor cells (23) with living antigenic variants of syngeneic tumor cells (24) may develop a potent specific CTL response that can be measured directly without in vitro stimulation. We have now adapted this approach and immunized DBA/2 mice intraperitoneally with syngeneic P815 cells transfected with the HLA-Cw3 gene.

The PELs exhibited a significant antigen-specific cytolytic activity against P815-Cw3 transfectant cells (Fig. 7A). Flow cytometric analysis showed that as many as 90% of the CD8+ PELs expressed V810-bearing TCRs (Fig. 7B). A second experiment confirmed these results and also showed that as few as 8% of the CD4+ PELs expressed V810-bearing TCRs (data not shown). Moreover, <8% of CD8+ PELs from nonimmunized DBA/2 mice or from C57BL/6 mice immunized with P815, expressed V810-bearing TCRs (data not shown).

The V810 junctional regions of freshly isolated PEL populations were amplified by cDNA-PCR using a V810-Cβ pair of primers. The PCR products were directly sequenced with a second antisense CB primer closer to the VDJC junction. The sequencing reaction was mostly readable in the junctional region, but became clearly readable in the V810 region, indicating that the vast majority of the V810-bearing cDNAs differed from the V83, A8 gene segments available upon request. All other segments but three are 100% identical at the nucleotide level to the published ones in the region sequenced, namely downstream of the Vβ primer used for PCR. The V83, F3.2 gene segment partial sequence in these DBA/2 clones differs from the original B6 sequence by a single base substitution; the V83, A8 gene segment partial sequence in BALB/c CTL clone Cw3/HLA2D3 differs from the V83, A8 gene segment in CTL clone Cw3/A8 by a single base substitution; and the V83, C9 gene segment partial sequence in clone Cw3/1F1 differs by a single base substitution from the original sequence. They are available upon request. The sequences are available in EMBL/GenBank DDBJ under accession number X66896.

TCR analysis of freshly isolated PELs specific for HLA-Cw3. The H-2-restricted T cell receptors specific for HLA-Cw3 were sequenced, namely downstream of the Vβ primer used for PCR. The V83, F3.2 gene segment partial sequence in these DBA/2 clones differs from the original B6 sequence by a single base substitution; the V83, A8 gene segment partial sequence in BALB/c CTL clone Cw3/HLA2D3 differs from the V83, A8 gene segment in CTL clone Cw3/A8 by a single base substitution; and the V83, C9 gene segment partial sequence in clone Cw3/1F1 differs by a single base substitution from the original sequence. They are available upon request. The sequences are available in EMBL/GenBank DDBJ under accession number X66896.
Table 1. Origin of H-2Kd-restricted HLA-Cw3-reactive CTL Clones Used in this Study*

| Mouse Strain | Immunogen† | In vitro stimulation | CTL clones | Reference |
|--------------|------------|----------------------|------------|-----------|
| 1 DBA/2      | P815-Cw3   | P815-Cw3             | Cw3/10.1   | 6         |
| 2 DBA/2      | P815-Cw3   | P815-Cw3             | Cw3/701.1  | 7         |
| 3 DBA/2      | P815-Cw3   | P815-Cw3             | Cw3/1.1, Cw3/56.1 | 7     |
| 4 DBA/2      | P815-Cw3   | P815-Cw3             | Cw3/2C1, Cw3/1F11 | This report |
| 5 DBA/2      | P815-Cw3   | P815 + peptide       | Cw3/A8     | This report |
| 6 DBA/2      | P815-Cw3sec| P815-Cw3sec          | Cw3/Cas1, Cw3/Cas2 | 28     |
|              |            |                      | Cw3/Cas3, Cw3/Cas7 |        |
|              |            |                      | Cw3/Cas15, Cw3/Cas20 |                |
| 7 DBA/2TgB7  | P815-Cw3   | P815-Cw3             | Cw3/4A3, Cw3/5B8 | 29†      |
| 8 DBA/2TgB7  | P815-Cw3/hβ2m| P815-Cw3/hβ2m     | Cw3/1B4    | 29†      |
| 9 DBA/2TgB7  | P815-Cw3/hβ2m| P815-Cw3/hβ2m     | Cw3/C37, Cw3/C44 | 29†     |
| 10 BALB/c   | peptide Cw3170-182 | P815 + peptide      | Cw3/HLA1C8, Cw3/HLA1G6 | This report** |
| 11 BALB/c   | peptide Cw3170-182 | P815 + peptide      | Cw3/HLA2A3, Cw3/HLA2D3 | This report** |

* The specificity of the CTL clones from mice 1 to 9 was established by recognition of the transfectant cell line P815-Cw3 (6) and was further documented (except Cw3/56.1, 1F11, Cas3, and C44) with P815 cells pulsed with synthetic peptides corresponding to the region 170–179 or 170–182 of the HLA-Cw3 molecule. The specificity of CTL clones from mice 10 and 11 was shown by recognition of the HLA-Cw3 170–179 peptide. In addition, a presumed sister clone of CTL clone Cw3/HLA1C8 was shown to kill P815-Cw3 cells as efficiently as the CTL clones isolated after immunization by the transfectant cell P185-Cw3. Clone HLA2D3 recognized P815-Cw3 less efficiently. Clones HLA1G6 and 2A3 were not tested against P815-Cw3. An additional CTL clone from mouse 11 recognized the Cw3 170–179 peptide, but was found not to express Vβ10 and is not included in this study. The H-2Kd restriction of the CTL clones from mice 1, 2, 3, and 5 is based on recognition of L cells transfected with H-2Kd, that of clones from mice 7 to 9 is based on antibody blocking experiments, and that of clones from mice 4, 6, 10, and 11 is presumed from recognition of Cw3 peptides which are known to bind to H-2Kd.

† P815-Cw3 indicates a P815 mastocytoma cell line transfected with the HLA-Cw3 gene (6), and P815-Cw3sec and P815-Cw3/hβ2m indicate P815 cell lines transfected with a gene encoding a secreted form of HLA-Cw3 (28) and a gene encoding human σ2 globulin in addition to the HLA-Cw3 gene (29), respectively.

‡ These clones were isolated as described in references 6 and 7.

§ CTL clone Cw3/A8 was derived as described (6, 7) except that P815 cells prepulsed with peptide Cw3 170–182 were used for in vitro stimulation.

¶ These CTL clones were not described in reference 29, but were derived in the reported experiments and found to be H-2Kd-restricted by antibody blocking experiments.

** BALB/c mice were immunized with peptide Cw3 170-182 in Freund's adjuvant as described in reference 27, and P815 cells pulsed with peptide Cw3 170-182 were used for in vitro stimulation.

97 were clearly readable, indicating that a Gly residue was also conserved among PELs specific for HLA-Cw3, as in all CTL clones. In contrast, direct sequencing with the same primer of Vβ10 PCR products of PELs from C57BL/6 mice immunized with P815 cells (Fig. 8) or from nonimmunized DBA/2 mice (data not shown) was not readable in any region. Altogether, the results indicated that CTLs in vivo were very similar in terms of TCR β chain structure to the CTL clones isolated and grown in vitro.

Discussion

We found that H-2Kd-restricted CTL clones specific for HLA-Cw3 within region 170–179 expressed TCRs of very limited diversity. All used the Vβ10 and JαPD158 segments, and all CDR3 α and β loops were found to display remarkably conserved features. This limited set of TCRs was selected despite differences among the mouse strains and immunization procedures used to derive these clones (Table 1). Moreover, the limited diversity of the TCRs of HLA-Cw3-reactive CTL clones established by in vitro culture is clearly representative of the TCR repertoire used in vivo, since PELs freshly isolated from mice immunized with HLA-Cw3 transfectant cells show the same TCR structures, not only in terms of Vβ10 segment usage, but also in terms of Vβ10-associated junctional region composition.

The HLA-Cw3-specific TCR repertoire clearly reflects an enrichment, since among unselected peripheral lymphocytes, <8% of CD8+ cells in DBA/2 mice are Vβ10 (10, and data not shown). Of the Vβ10-bearing TCRs (25, 26) and JαPD158-bearing TCRs (5, 19) reported in the literature, none resembles those specific for HLA-Cw3. Moreover, out of 54 additional independent H-2Kd-restricted CTL clones specific for antigens unrelated to HLA-Cw3, only two were
found to be Vβ10, and their junctional regions differ considerably from the HLA-Cw3-reactive Vβ10 chains (Casanova et al., manuscript in preparation).

The very limited heterogeneity of the TCRs specific for the HLA-Cw3 peptide stands in marked contrast to our previous report that the TCRs of CTLs specific for a PbCS peptide showed highly diverse primary structures (5). It is notable that both studies are comparable with respect to immunization procedures, mouse strains, and restriction element. In addition, both peptides bind to H-2Kd with a similar affinity (8, and data not shown). As a possible explanation for the difference in the TCR repertoire size, the HLA-Cw3 peptide is one amino acid longer than the PbCS peptide. However, this increased number of solvent-accessible side chains between the two residues “anchoring” the peptides to H-2Kd (8) should rather expose on the surface of the complex more epitopes available for TCR contact. More likely, a feature that may account for the TCR repertoire size differences between the two systems seems to be that, unlike the PbCS peptide, the HLA-Cw3 peptide is homologous to self peptides derived from the same region of the H-2 molecules. It is significant that a survey of the previously reported class II MHC-restricted TCR repertoires supports this hypothesis (5).

In this model, among the T cell epitopes potentially displayed by the HLA-Cw3 peptide/H-2Kd molecule complex, a significant degree of overlap would be expected to occur with those displayed by self H-2 peptide/H-2Kd molecule complexes. As a consequence of natural tolerance to self epitopes, a significant fraction of CTLs potentially HLA-Cw3 reactive may not be recruited during the immune response. In support of this view, when tested for recognition of the HLA-Cw3 peptide in the context of a series of genetically engineered mutants of the H-2Kd molecule, all CTL clones tested so far displayed the same recognition pattern (Maryanski et al., manuscript in preparation). This stands in contrast to the highly diverse recognition patterns we have previously

### Table 1

| CTL clone | Vβ | FW | CDR3 | Jβ |
|-----------|----|----|------|----|
| Cw3/1.1   | 10 | CAS SLGS D Y | TFG 1.2 |
| Cw3/2A1   | 10 | CAS SLGS D Y | TFG 1.2 |
| Cw3/Cas20 | 10 | CAS S RGS D Y | TFG 1.2 |
| Cw3/4A3   | 10 | CAS S RGS D Y | TFG 1.2 |
| Cw3/HLA1G6| 10 | CAS S RGS D Y | TFG 1.2 |
| Cw3/Cas2  | 10 | CAS S FGS D Y | TFG 1.2 |
| Cw3/C44   | 10 | CAS S FGS D Y | TFG 1.2 |
| Cw3/HLA1C8| 10 | CAS S QGT D Y | TFG 1.2 |
| Cw3/C37   | 10 | CAS S QGT D Y | TFG 1.2 |
| Cw3/HLA2D3| 10 | CAS S GTD Y | TFG 1.2 |
| Cw3/10.1  | 10 | CAS S GFD T Y | TFG 1.2 |
| Cw3/Cas3  | 10 | CAS S WGG Y | TFG 1.2 |
| Cw3/A8    | 10 | CAS S LGET L | TFG 2.3 |
| Cw3/Cas1  | 10 | CAS S LGET L | TFG 2.3 |
| Cw3/1F11  | 10 | CAS S PGET L | TFG 2.3 |
| Cw3/701.1 | 10 | CAS S GET L | TFG 2.3 |
| Cw3/56.1  | 10 | CAS S WGET L | TFG 2.3 |
| Cw3/Cas7  | 10 | CAS S GCR L | TFG 1.4 |
| Cw3/1B4   | 10 | CAS S QCR L | TFG 1.4 |
| Cw3/2C3   | 10 | CAS S ECR L | TFG 1.4 |
| Cw3/HLA2D3| 10 | CAS S SGRVE | TFG 2.7 |
| Cw3/5B8   | 10 | CAS S KSV GY | TFG 2.7 |
| Cw3/Cas15 | 10 | CAS S FQG EV | TFG 1.1 |

### Table 2

| CTL clone | Vα | FW | CDR3 | Jα |
|-----------|----|----|------|----|
| Cw3/1.1   | 8  | p71 | CAL SEG GFA SAL | TFG pHDS58 |
| Cw3/HLA2A3| 8  | p71 | CAL SEG GFA SAL | TFG pHDS58 |
| Cw3/Cas20 | 8  | p71 | CAL SDQ GFA SAL | TFG pHDS58 |
| Cw3/Cas15 | 8  | p71 | CAL SDQ GFA SAL | TFG pHDS58 |
| Cw3/Cas2  | 8  | F3.2 | CAL DRQ GFA SAL | TFG pHDS58 |
| Cw3/Cas7  | 8  | F3.2 | CAL SGR GFA SAL | TFG pHDS58 |
| Cw3/Cas3  | 8  | F3.4 | CAL SEG GFA SAL | TFG pHDS58 |
| Cw3/C57   | 8  | F3.6 | CAL SDR QFA SAL | TFG pHDS58 |
| Cw3/2C1   | 4  | TAA55 | CAL SDR QFA SAL | TFG pHDS58 |
| Cw3/C44   | 4  | TAA55 | CAL SDR QFA SAL | TFG pHDS58 |
| Cw3/HLA1C8| 4  | CAL GDP GFA SAL | TFG pHDS58 |
| Cw3/5B8   | 4  | CAL GEG QFA SAL | TFG pHDS58 |
| Cw3/Cas3  | 4  | CAL GDF QFA SAL | TFG pHDS58 |
| Cw3/Cas15 | 4  | CAL GDF QFA SAL | TFG pHDS58 |

### Figure 5

TCR β chain junctional region amino acid sequences. (Vertical axis) The 23 CTL clones. For each clone, the in-frame TCR β transcript encoding the key residues at the VDJ junction (22) was considered to encode the functional TCR β chain. For CTL clones Cw3/1.1, 10.1, 701.1, A8, Cas1, and Cas20, a FACS® staining with the antiVβ10 mAb B2.13 (10) was performed to confirm the β transcript assignment. The deduced amino acid sequences of the junctional and hypervariable region, putatively CDR3-like, are reported in single-letter amino acid code (22). The presumed Ig-like loop, designated CDR3 for convenience, is putatively supported by two framework branches (FW). The key cysteine residue is at position 92 in the β chain. The Vβ and Jβ segments are also reported (see legend to Fig. 4 for references).

### Figure 6

TCR α chain junctional region amino acid sequences. (Vertical axis) The 23 CTL clones. Because T cells may display two productive α rearrangements (5, 41), the functional α chains, engaged in heterodimeric formation with the β chain and specific for the HLA-Cw3 peptide/H-2Kd molecule complex, can be rigorously determined only for CTL clones in which a second, out-of-frame, α gene or transcript is identified. In CTL clone Cw3/1.1, both α rearrangements were sequenced, and only one was found to be productive (Figs. 2 and 3). In CTL clones Cw3/701.1, Cas1, 4A3, C44, 2C1, and HLAIC8, a second, out-of-frame, α transcript was detected by cDNA-PCR (data not shown). A second transcript, in-frame at the VJ junction, was detected in CTL clone Cw3/HLA2D3 (data not shown). Nevertheless, for the latter and the remaining CTL clones, the JαHDS58-bearing rearrangements are likely to encode the functional α chains, considering that their structures are very similar to the unambiguously assigned ones. The deduced amino acid sequences of the junctional and hypervariable region, putatively CDR3-like, are reported in single-letter amino acid code (22). The presumed Ig-like loop, designated CDR3 for convenience, is putatively supported by two framework branches (FW). The key cysteine is at position 90 in the α chain. The Vα and Jα segments are also reported (see Fig. 4 for references).
observed with PbCS-specific CTL clones tested for recognition either of the PbCS peptide in the context of mutant H-2K^d^ molecules (Jaulin, C., J.-L. Casanova, P. Romero, I. Gueschen, A.-S. Cordey, J. L. Maryanski and P. Kourilsky, manuscript submitted for publication) or of variant PbCS peptides in the context of H-2K^d^ (27). It suggests that the former CTL clones interact with fewer distinct epitopes on the surface of the MHC-peptide complex than the latter.

Altogether, our study establishes that the presentation by H-2K^d^ of a peptide from HLA-Cw3 results in the selection of CTLs that express TCRs of very limited diversity, not only in vitro, but also in vivo. This supports the possibility that antigenic peptides homologous to host-derived peptides trigger T cells of limited TCR diversity. Conversely, T cell responses against peptides heterologous to the host, such as those derived from P. berghei or from other infectious agents, would be highly diverse. Such a diversity may favor the host, not only by increasing the potency of the response, but also by reducing the likelihood of the survival and escape of antigenic variants of the pathogen.

We thank G. Corradin, P. Kourilsky, and M. Malissen for advice and discussion; J. A. Lopez, P. Romero, and A. Wilson for critical reading of the manuscript; C. Gregoire for Southern blot analysis; A. Millward for constructing the Cw3/1.1 genomic library; G. Miescher for purification of Cw3/1.1 DNA; C. Horvath, A.-L. Peitrequin, and G. Bieler for excellent technical assistance; and C. Knabenhans and P. Zaech for FACS\textsuperscript{a} analysis. Jean-Laurent Casanova wishes to express his gratitude to Bruno Varet for his continuing help, advice, and encouragement.

Jean-Laurent Casanova was supported by the Institut National de la Santé et de la Recherche Médicale (INSERM), and by the European Organization for the Research on the Treatment of Cancer; MichaelaMattthes by the Fondation pour la Recherche Médicale; and Antje Necker by the Deutsche Forschungsgemeinschaft. This work was supported in part by institutional grants from the Centre National de la Recherche Scientifique and INSERM.

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Received for publication 4 February 1992.
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