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

By Jean-Laurent Casanova,* Jean-Charles Cerottini,* Michaela Matthes,‡ Antje Necker,‡ Hélène Gournier,‡ Claude Barra,‡ Christian Widmann,§ H. Robson MacDonald,* François Lemonnier,‡ Bernard Malissen,‡ and Janet L. Maryanski*

*From the *Ludwig Institute for Cancer Research, Lausanne Branch, 1066 Epalinges, Switzerland; the ‡Centre d'Immunologie de Marseille-Luminy, 13288 Marseille Cedex 9, France; and the §Institut de Biochimie, Université de Lausanne, 1066 Epalinges, Switzerland*

Summary

We previously showed that H-2K^d–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-2K^d. We first sequenced the TCR α and β genes of the CTL clone Cw3/1.1 and, based on this genomic analysis, the TCR α and β cDNA junctional regions of 23 independent H-2K^d–restricted CTL clones specific for HLA-Cw3. The results show that the TCR chains display very limited heterogeneity, both in terms of Vα, Jα, Vβ, and Jβ segments, and in terms of length and sequence of the CDR3 α 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-2K^d 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 $\alpha\beta$ 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-2K^d–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 Vβ segment dominance (5).

We have now analyzed the TCR repertoire of H-2K^d–restricted CTLs specific for P815 (H-2^d) 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-2^d molecules.

We found that the TCRs carried by H-2K^d–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.

J. Exp. Med. © The Rockefeller University Press • 0022-1007/92/08/0439/09 $2.00
Volume 176 August 1992 439–447

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-2^d) mice were injected intraperitoneally with 2.5×10^7 unirradiated P815 cells transfected with the HLA-Cw3 gene (6). A second injection with 10^7 cells was performed 5 wk later. Control C57BL/6 (H-2^b) 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 FACScan® 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, Cα, 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-^{35}S-dATP was carried out as originally described (14) with minor modifications (15).

Results

To analyze the TCR repertoire of H-2K^d–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

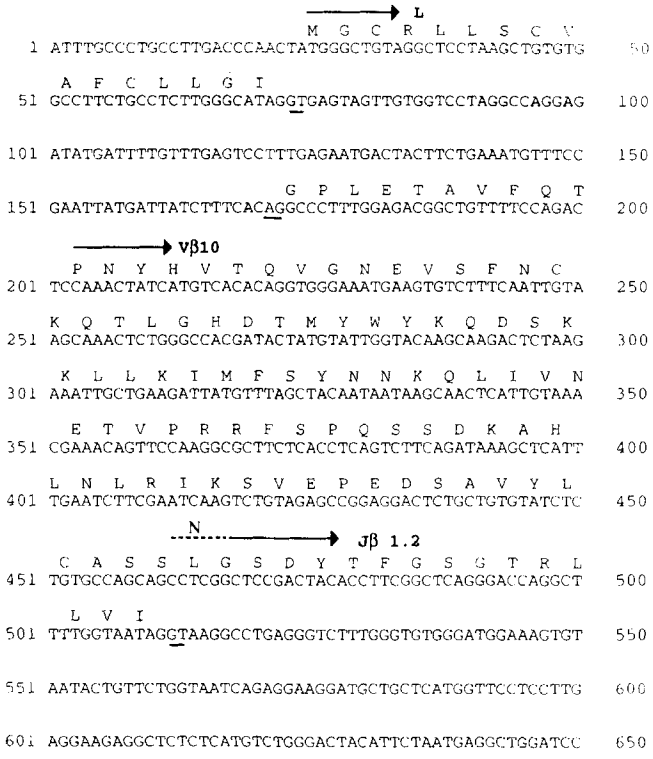

Figure 1. Sequence of the productive TCR β gene rearrangement found in CTL clone Cw3/1.1. The predicted amino acid sequence is shown above the nucleotide sequence. (*Horizontal arrows*) Boundaries between regions. (*N*) Probable N-region sequences. RNA splicing sites are underlined. This sequence is available from EMBL/GenBank/DDBJ under accession number X66896.

of the Dδ-Jδ-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

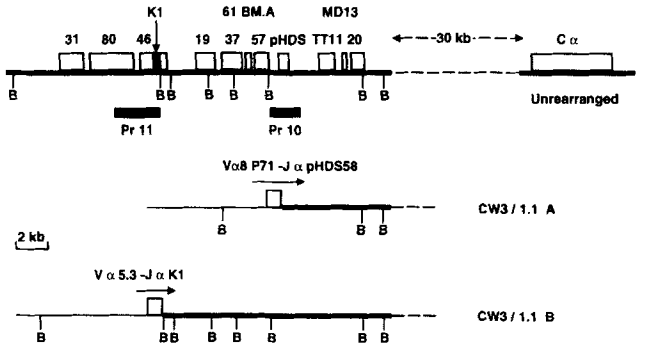

Figure 2. Structure of the two TCR α gene rearrangements in CTL clone Cw3/1.1. Partial restriction maps of the two alleles (*Cw3/1.1A* and *Cw3/1.1 B*) are compared with the corresponding region of the unrearranged TCR α gene locus. (*Open boxes*) Exons encoding Jα gene segments and Vα and Cα genes. The positions of previously identified Jα gene segments are indicated according to references 12, 30, and 31. (*Vertical arrow*) Location of the Jα K1 gene segment (see text). (*Horizontal solid arrows*) 5′ to 3′ orientation. (*Vertical lines*) Positions of the relevant BamHI (*B*) restriction sites. (*Solid boxes*) Position of the Jα single copy probes Pr 10 and Pr 11 (12).

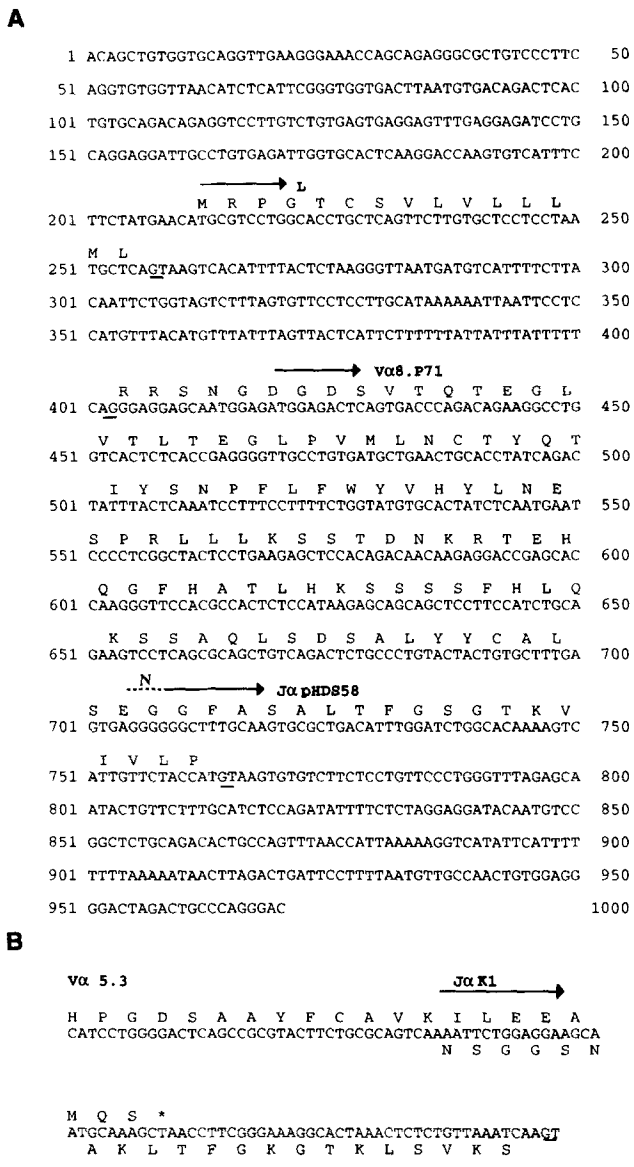

Figure 3. Sequence of the TCR α gene rearrangements in CTL clone Cw3/1.1. (*A*) Sequence of the productive rearrangement Cw3/1.1 A. The predicted amino acid sequence encoded by the exons is shown above the nucleotide sequence. (*Horizontal arrows*) Boundaries between regions. (*L*) Leader. The 5′ boundary of the Jα pHDS58 segment was determined by the analysis of the corresponding germline sequence (20). The putative 3′ boundary of Vα8.p71 is deduced from the analysis of TCR α cDNAs involving Vα segments related to the Vα8.P71 sequences (18, 32). (N) Probable N-region nucleotides. RNA splicing donor and acceptor sites are underlined. (*B*) Partial nucleotide sequence of the nonproductive rearrangement Cw3/1.1 B. Two amino acid sequences are indicated for the JαK1 nucleotide sequence. (*Top*) Amino acid sequence corresponds to that resulting from the nonproductive V-J joining observed in Cw3/1.1 cells. (*Bottom*) Amino acid sequence corresponds to that expected from a productive rearrangement. (*) Premature termination codon observed in the Cw3/1.1 B rearrangement. These sequences are available from EMBL/GenBank/DDBJ under accession number X66896.

Vα8 subfamily, whose coding region was 100% identical at the nucleotide level to the BALB/c Vαp71 gene segment (18), and the JαpHDS58 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 Vα5 subfamily with a coding region 100% identical at the nucleotide level to the VαMDA gene segment (18), referred to as Vα 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-2K^d–restricted CTL clones specific for HLA-Cw3 was submitted to cDNA-PCR with a sense Vβ10 primer in conjunction with an antisense Cβ 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 animal of origin (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 Vα8 primer in conjunction with an antisense Cα primer. Eight clones expressed a Vα8 transcript and the remaining 15 clones were tested sequentially with primers corresponding to most Vα 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 Vα subfamilies, only five were found and three of these, namely Vα3, Vα4, and Vα8, 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. Several pairs of CTL clones apparently bearing identical TCR α chain amino acid sequences were found, although additional diversity in the nonsequenced part of the Vα segment could not be rigorously excluded. Many other clones differed in the CDR3 junctional region only, at a single or a few positions, often by conservative substitutions. Some clones shared identical CDR3 sequences, but differed in Vα segment usage.

A TCR α cDNAs

CTL clone	Vα		Jα
Cw3/1.1	8.p71	...TGT GCT TTG AGT GAG GGG GGC TTT GCA AGT GCG CTG...	pHDS58
Cw3/HLA2A3	8.p71	...TGT GCT TTG AGT GAA GGA GGC TTT GCA AGT GCG CTG...	pHDS58
Cw3/Cas20	8.p71	...TGT GCT TTG AGT GAT CAG GGC TTT GCA AGT GCG CTG...	pHDS58
Cw3/Cas15	8.p71	...TGT GCT TTG AGT GAT GGG GGC TTT GCA AGT GCG CTG...	pHDS58
Cw3/Cas2	8.F3.2	...TGT GCT TTG AGT GAT CGA GGC TTT GCA AGT GCG CTG...	pHDS58
Cw3/Cas7	8.F3.2	...TGT GCT TTG AGT GAA CGG GGC TTT GCA AGT GCG CTG...	pHDS58
Cw3/Cas3	8.F3.4	...TGT GCT TTG AGT GAA GGG GGC TTT GCA AGT GCG CTG...	pHDS58
Cw3/C37	8.F3.6	...TGT GCT CTG AGT GAT CGA GGC TTT GCA AGT GCG CTG...	pHDS58
Cw3/2C1	4.TA65	...TGT GCT CTG AGT GAT CGG GGC TTT GCA AGT GCG CTG...	pHDS58
Cw3/C44	4.TA65	...TGT GCT CTG AGT GAT CGG GGC TTT GCA AGT GCG CTG...	pHDS58
Cw3/HLA1C8	4.3	...TGT GCT CTG GGT GAC CCA GGC TTT GCA AGT GCG CTG...	pHDS58
Cw3/5B8	4.3	...TGT GCT CTG GGT GAA GGG GGC TTT GCA AGT GCG CTG...	pHDS58
Cw3/HLA1G6	4.1G6	...TGT GCT CTG GGT GAT CGG GGC TTT GCA AGT GCG CTG...	pHDS58
Cw3/4A3	4.PJR25	...TGC GCT CTG AGT GAT CGG GGC TTT GCA AGT GCG CTG...	pHDS58
Cw3/A8	3.A8	...TGT GCT CTG AGC ATG GGA GGC TTT GCA AGT GCG CTG...	pHDS58
Cw3/HLA2D3	3.A8	...TGT GCT GTG AGC GCG GGA GGC TTT GCA AGT GCG CTG...	pHDS58
Cw3/56.1	3.AR-5	...TGT GCT CTG AGC GCG ACA GGC TTT GCA AGT GCG CTG...	pHDS58
Cw3/1B4	3.AR-5	...TGT GCT CTG AGC GCG ACA GGC TTT GCA AGT GCG CTG...	pHDS58
Cw3/701.1	3.AR-5	...TGT GCT CTG ACC CAG ACA GGC TTT GCA AGT GCG CTG...	pHDS58
Cw3/1F11	3.C9	...TGT GCT GTG AGC GAG ACA GGC TTT GCA AGT GCG CTG...	pHDS58
Cw3/Cas1	5.MDA	...TGC GCA GTC AGT GCG GGG GGC TTT GCA AGT GCG CTG...	pHDS58
Cw3/2C3	5.MDA	...TGC GCA GTC AGT GAG GGG GGC TTT GCA AGT GCG CTG...	pHDS58
Cw3/10.1	1.TA84	...TGT GCA GTT AGT GAG CAC GGC TTT GCA AGT GCG CTG...	pHDS58

B TCR β cDNAs

CTL clone	Vβ		Jβ
Cw3/1.1	10	...TGT GCC AGC AGC CTC GGC TCC GAC TAC ACC TTC GGC...	1.2
Cw3/2C1	10	...TGT GCC AGC AGC TTG GGC TCC GAC TAC ACC TTC GGC...	1.2
Cw3/Cas20	10	...TGT GCC AGC AGC CTA GGA TCC GAC TAC ACC TTC GGC...	1.2
Cw3/4A3	10	...TGT GCC AGC AGC AGA GGC TCC GAC TAC ACC TTC GGC...	1.2
Cw3/HLA1G6	10	...TGT GCC AGC AGC CGG GGC TCC GAC TAC ACC TTC GGC...	1.2
Cw3/Cas2	10	...TGT GCC AGC AGC TTT GGC TCC GAC TAC ACC TTC GGC...	1.2
Cw3/C44	10	...TGT GCC AGC AGC CAG GGC TCC GAC TAC ACC TTC GGC...	1.2
Cw3/HLA1C8	10	...TGT GCC AGC AGC CAG GGA ACC GAC TAC ACC TTC GGC...	1.2
Cw3/C37	10	...TGT GCC AGC AGC TAC GGG ACC GAC TAC ACC TTC GGC...	1.2
Cw3/HLA2D3	10	...TGT GCC AGC AGC TAC GGG ACC GAC TAC ACC TTC GGC...	1.2
Cw3/10.1	10	...TGT GCC AGC AGC ACA GGG TTC GAC TAC ACC TTC GGC...	1.2
Cw3/Cas3	10	...TGT GCC AGC AGC TGG GGA CAG GGC TAC ACC TTC GGC...	1.2
Cw3/A8	10	...TGT GCC AGC AGC TTG GGA GAA ACG CTG TAT TTT GGC...	2.3
Cw3/Cas1	10	...TGT GCC AGC AGC TTG GGG GAA ACG CTG TAT TTT GGC...	2.3
CW3/1F11	10	...TGT GCC AGC AGC TTT GGG GAA ACG CTG TAT TTT GGC...	2.3
Cw3/701.1	10	...TGT GCC AGC AGC TAC GGC GAA ACG CTG TAT TTT GGC...	2.3
Cw3/56.1	10	...TGT GCC AGC AGC TGG GGG GAA ACG CTG TAT TTT GGC...	2.3
Cw3/Cas7	10	...TGT GCC AGC AGC TTC GGC GAA AGA TTA TTT TTC GGT...	1.4
Cw3/1B4	10	...TGT GCC AGC AGC CAG GGG GAA AGA TTA TTT TTC GGT...	1.4
Cw3/2C3	10	...TGT GCC AGC AGC TAT GGG GAA AGA TTA TTT TTC GGT...	1.4
Cw3/HLA2A3	10	...TGT GCC AGC AGC TCC GGG AGG GTG GAG TAC TTC GGT...	2.7
Cw3/5B8	10	...TGT GCC AGC AGC AAG GGG GTT ATG GGG TAC TTC GGT...	2.7
Cw3/Cas15	10	...TGT GCC AGC AGC TTC GGA CAA GAA GTC TTC TTT GGT...	1.1

Figure 4. TCR α and β cDNA junctional nucleotides sequences. (*A*) TCR α junctional sequences. The nomenclature for the Vα gene subfamilies follows that of references 4, 5, and 33. In the figure, 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: 18 (*Vα8.p71* and *Vα5.MDA*); 32 (*Vα8.F3.2*, *Vα8.F3.4*, and *Vα8.F3.6*); 21 (*Vα4.TA65* and *Vα1.TA84*); 34 (*Vα4.3*); 35 (*Vα4.PJR-25*); 36 (*Vα3.AR-5*); 37 (*Vα3.C9*), and this report (*Vα4.1G6* and *Vα3.A8*). Because of partial sequencing, except for the CTL clone Cw3/1.1, the Vα segment identification is only putative. The partial sequences of the Vα4.1G6 and Vα3.A8 gene segments are 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 Vα primer used for PCR. The Vα8.F3.2 gene segment partial sequence in these DBA/2 clones differs from the original B6 sequence by a single base substitution; the Vα3.A8 gene segment partial sequence in BALB/c CTL clone Cw3/HLA2D3 differs from the Vα3.A8 gene segment in CTL clone Cw3/A8 by a single base substitution; and the Vα3.C9 gene segment partial sequence in clone Cw3/1F11 differs by a single base substitution from the original sequence. They are available upon request. The JαpHDS58 gene segment is from references 19, 20. (*B*) TCR β cDNA junctional sequences. The nomenclature for the functional Vβ gene segments of the $V\beta^b$ haplotype follows that of references 4 and 5. The Vβ10 sequence was originally described in reference 16. The Jβ gene segments sequences are from references 17, 38, except Jβ1.4, which is from reference 5. Unambiguously assigned Dβ gene segments (39, 40) are underlined. These sequences are available from EMBL/GenBank DDBJ under accession number X66896.

TCR Analysis of Freshly Isolated PELs Specific for HLA-Cw3. It has been shown previously that mice injected intraperitoneally with allogeneic irradiated tumor cells (23) or 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. 7 *A*). Flow cytometric analysis showed that as many as 90% of the $CD8^+$ PELs expressed Vβ10-bearing TCRs (Fig. 7 *B*). A second experiment confirmed these results and also showed that as few as 8% of the $CD4^+$ PELs expressed Vβ10-bearing TCRs (data not shown). Moreover, <8% of $CD8^+$ PELs from nonimmunized DBA/2 mice or from C57BL/6 mice immunized with P815, expressed Vβ10-bearing TCRs (data not shown).

The Vβ10 junctional regions of freshly isolated PEL populations were amplified by cDNA-PCR using a Vβ10-Cβ pair of primers. The PCR products were directly sequenced with a second antisense Cβ primer closer to the VDJC junction. The sequencing reaction was mostly unreadable in the junctional region, but became clearly readable in the Vβ10 region, indicating that the vast majority of the Vβ10-bearing cDNAs differed in their junctional sequence but displayed the same junctional length (Fig. 8). Remarkably, these junctional regions were found to have precisely the same length in nucleotides as those from the HLA-Cw3–reactive CTL clones. Thus, they must also encode six amino acid-long CDR3 loops. In addition, of the nucleotides in the junctional region, two successive guanine nucleotides (G) in the first two positions of the triplet encoding the amino acid residue

Table 1. *Origin of H-2K^d–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 Cw3/2C3	This report§
5	DBA/2	P815-Cw3	P815 + peptide	Cw3/A8	This report‖
6	DBA/2	P815-Cw3*sec*	P815-Cw3*sec*	Cw3/Cas1, Cw3/Cas2 Cw3/Cas3, Cw3/Cas7 Cw3/Cas15, Cw3/Cas20	28
7	DBA/2*TgB7*	P815-Cw3	P815-Cw3	Cw3/4A3, Cw3/5B8	29¶
8	DBA/2*TgB7*	P815-Cw3/$h\beta_2m$	P815-Cw3/$h\beta_2m$	Cw3/1B4	29¶
9	DBA/2*TgB7*/$h\beta_2m$	P815-Cw3/$h\beta_2m$	P815-Cw3/$h\beta_2m$	Cw3/C37, Cw3/C44	29¶
10	BALB/c	peptide $Cw3_{170-182}$	P815 + peptide	Cw3/HLA1C8, Cw3/HLA1G6	This report**
11	BALB/c	peptide $Cw3_{170-182}$	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-2K^d restriction of the CTL clones from mice 1, 2, 3, and 5 is based on recognition of L cells transfected with H-2K^d, 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-2K^d.

‡ P815-Cw3 indicates a P815 mastocytoma cell line transfected with the HLA-Cw3 gene (6), and P815-Cw3*sec* and P815-Cw3/$h\beta_2m$ indicate P815 cell lines transfected with a gene encoding a secreted form of HLA-Cw3 (28) and a gene encoding human β_2 microglobulin 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-2K^d–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-2K^d–restricted CTL clones specific for HLA-Cw3 within region 170–179 expressed TCRs of very limited diversity. All used the Vβ10 and JαpHDS58 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 JαpHDS58-bearing TCRs (5, 19) reported in the literature, none resembles those specific for HLA-Cw3. Moreover, out of 54 additional independent H-2K^d–restricted CTL clones specific for antigens unrelated to HLA-Cw3, only two were

CTL clone	Vβ	FW	CDR3	FW	Jβ
Cw3/1.1	10	CAS	S L G S D Y	TFG	1.2
Cw3/2C1	10	CAS	S L G S D Y	TFG	1.2
Cw3/Cas20	10	CAS	S L G S D Y	TFG	1.2
Cw3/4A3	10	CAS	S R G S D Y	TFG	1.2
Cw3/HLA1G6	10	CAS	S R G S D Y	TFG	1.2
Cw3/Cas2	10	CAS	S F G S D Y	TFG	1.2
Cw3/C44	10	CAS	S Q G S D Y	TFG	1.2
Cw3/HLA1C8	10	CAS	S Q G T D Y	TFG	1.2
Cw3/C37	10	CAS	S Y G T D Y	TFG	1.2
Cw3/HLA2D3	10	CAS	S Y G T D Y	TFG	1.2
Cw3/10.1	10	CAS	S T G F D Y	TFG	1.2
Cw3/Cas3	10	CAS	S W G Q G Y	TFG	1.2
Cw3/A8	10	CAS	S L G E T L	YFG	2.3
Cw3/Cas1	10	CAS	S L G E T L	YFG	2.3
Cw3/1F11	10	CAS	S F G E T L	YFG	2.3
Cw3/701.1	10	CAS	S Y G E T L	YFG	2.3
Cw3/56.1	10	CAS	S W G E T L	YFG	2.3
Cw3/Cas7	10	CAS	S F G E R L	FFG	1.4
Cw3/1B4	10	CAS	S Q G E R L	FFG	1.4
Cw3/2C3	10	CAS	S Y G E R L	FFG	1.4
Cw3/HLA2A3	10	CAS	S S G R V E	YFG	2.7
Cw3/5B8	10	CAS	S K G V M G	YFG	2.7
Cw3/Cas15	10	CAS	S F G Q E V	FFG	1.1

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 B21.5 (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).

CTL clone	Vα	FW	CDR3	FW	Jα
Cw3/1.1	8.p71	CAL	S E G G F A S A L	TFG	pHDS58
Cw3/HLA2A3	8.p71	CAL	S E G G F A S A L	TFG	pHDS58
Cw3/Cas20	8.p71	CAL	S D Q G F A S A L	TFG	pHDS58
Cw3/Cas15	8.p71	CAL	S D G G F A S A L	TFG	pHDS58
Cw3/Cas2	8.F3.2	CAL	S D R G F A S A L	TFG	pHDS58
Cw3/Cas7	8.F3.2	CAL	S E R G F A S A L	TFG	pHDS58
Cw3/Cas3	8.F3.4	CAL	S E G G F A S A L	TFG	pHDS58
Cw3/C37	8.F3.6	CAL	S D R G F A S A L	TFG	pHDS58
Cw3/2C1	4.TA65	CAL	S D R G F A S A L	TFG	pHDS58
Cw3/C44	4.TA65	CAL	S D R G F A S A L	TFG	pHDS58
Cw3/HLA1C8	4.3	CAL	G D P G F A S A L	TFG	pHDS58
Cw3/5B8	4.3	CAL	G E G G F A S A L	TFG	pHDS58
Cw3/HLA1G6	4.1G6	CAL	G D R G F A S A L	TFG	pHDS58
Cw3/4A3	4.PJR-25	CAL	S D R G F A S A L	TFG	pHDS58
Cw3/A8	3.A8	CAL	S M G G F A S A L	TFG	pHDS58
Cw3/HLA2D3	3.A8	CAV	S A G G F A S A L	TFG	pHDS58
Cw3/56.1	3.AR-5	CAL	S A T G F A S A L	TFG	pHDS58
Cw3/1B4	3.AR-5	CAL	S A T G F A S A L	TFG	pHDS58
Cw3/701.1	3.AR-5	CAL	T Q T G F A S A L	TFG	pHDS58
Cw3/1F11	3.C9	CAV	S E T G F A S A L	TFG	pHDS58
Cw3/Cas1	5.MDA	CAV	S A G G F A S A L	TFG	pHDS58
Cw3/2C3	5.MDA	CAV	S E G G F A S A L	TFG	pHDS58
Cw3/10.1	1.TA84	CAV	S E H G F A S A L	TFG	pHDS58

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 chains and specific for the HLA-Cw3 peptide/H-2K^d 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 HLA1C8, a second, out-of-frame, α transcript was detected by cDNA-PCR (data not shown). A second transcript, in-frame at the V-J junction, was detected in CTL clone Cw3/HLA2D3 (data not shown). Nevertheless, for the latter and the remaining CTL clones, the JαpHDS58-bearng 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).

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-2K^d 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-2K^d (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-2K^d molecule complex, a significant degree of overlap would be expected to occur with those displayed by self H-2 peptide/H-2K^d 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-2K^d 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

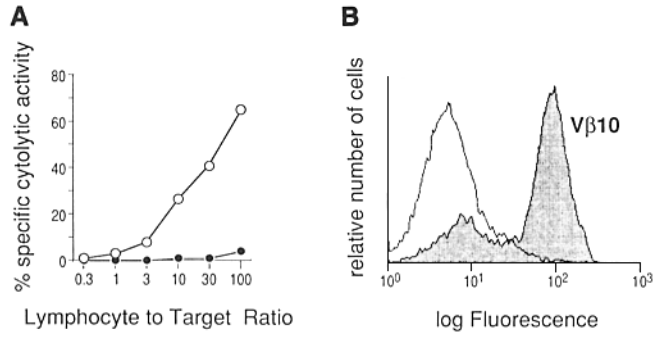

Figure 7. Specific cytolytic activity and Vβ FACS® analysis of freshly isolated PELs from DBA/2 mice immunized with P815-Cw3 transfectant cells. (*A*) Nylon wool purified PELs were tested for recognition of ^{51}Cr-labeled P815-Cw3 (*open circles*) and untransfected P815 (*closed circles*) cells, at various E/T ratios. (*B*) the PELs were double-stained with mAbs against CD8 and Vβ10. (*Shaded histogram*) Vβ10 staining of cells that have been gated as CD8$^+$. (*Solid line*) Control staining of CD8$^+$ cells with the FITC conjugate alone.

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.

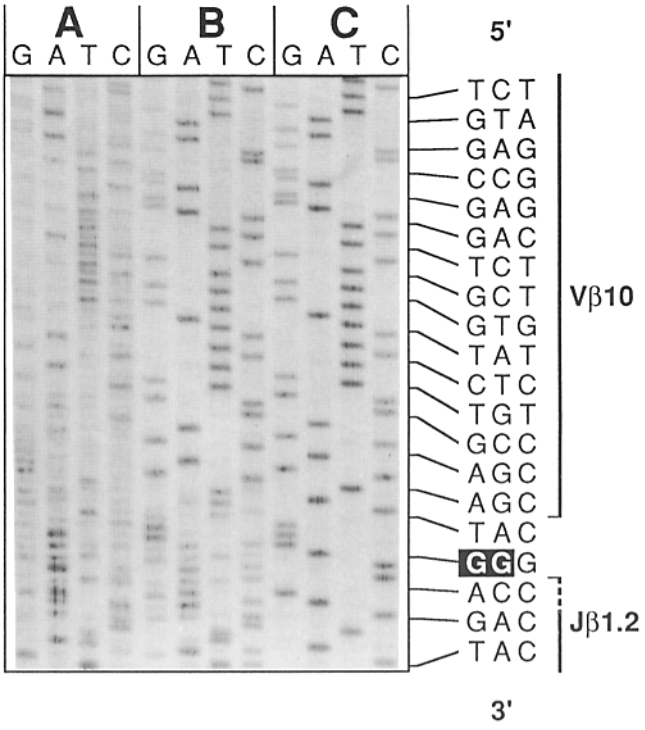

Figure 8. Limited sequence heterogeneity of Vβ10-bearing TCR β cDNA junctional regions of freshly isolated PELs. PELs originating from DBA/2 mice immunized with P815-Cw3 transfectant cells and from C57BL/6 mice immunized with untransfected P815 cells were purified by passage over nylon wool. RNA was extracted from the PEL samples and from the CTL clone Cw3/HLA2D3, as a control. After cDNA synthesis the Vβ10 junctional regions were amplified by PCR using a combination of Vβ10 and Cβ primers. The noncoding strand of each double-stranded PCR product was directly sequenced using a second antisense Cβ primer closer to the VDJ junction. The three samples (*A*, *C57BL/6* PEL-s; *B*, DBA/2 PELs; and *C*, clone Cw3/HLA2D3) were loaded on the same sequencing gel. For convenience, the autoradiogram is shown backwards and the complementary nucleotides are indicated, in the GATC order, to allow reading of the coding strand (from top to bottom). The sequence for the control CTL clone is written at the side. The nucleotides corresponding to the Vβ10 and Jβ1.2 segments, and those encoding the conserved Gly at position 97, are indicated.

We thank G. Corradin, P. Kourilsky, and M. Malissen for advice and discussion; J. A. Lòpez, 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® 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; Michaela Matthes by the Fondation pour la Recherche Médicale; and Antje Necker by the Deutsche Forchungsgemeinschaft. This work was supported in part by institutional grants from the Centre National de la Recherche Scientifique and INSERM.

Address correspondence to Jean-Laurent Casanova, Ludwig Institute for Cancer Research, Lausanne Branch, 1066 Epalinges, Switzerland.

Received for publication 4 February 1992.

References

1. Rötzschke, O., and K. Falk. 1991. Naturally-occurring peptide antigens derived from the MHC class I-restricted processing pathway. *Immunol. Today.* 12:447.
2. Townsend, A., and H. Bodmer. 1989. Antigen recognition by class I restricted T lymphocytes. *Annu. Rev. Immunol.* 7:601.
3. Matis, L. 1990. The molecular basis of T cell specificity. *Annu. Rev. Immunol.* 8:65.
4. Wilson, R.K., E. Lai, P. Concannon, R.K. Barth, and L.E. Hood. 1988. Structural organization and polymorphism of murine and human T cell receptor α and β gene families. *Immunol. Rev.* 101:149
5. Casanova, J.-L., P. Romero, C. Widmann, P. Kourilsky, and J.L. Maryanski. 1991. T cell receptor genes in a series of class I major histocompatibility complex-restricted cytotoxic T lymphocyte clones specific for a single *Plasmodium berghei* nonapeptide. Implications for T cell allelic exclusion and antigen-specific repertoire. *J. Exp. Med.* 174:1371.
6. Maryanski, J.L., R.S. Accolla, and B.R. Jordan. 1986. H-2 restricted recognition of cloned HLA class I gene products expressed in mouse cells. *J. Immunol.* 136:4340.
7. Pala, P., G. Corradin, T. Strachan, R. Sodoyer, B. Jordan, J.-C. Cerottini, and J.L. Maryanski. 1988. Mapping of HLA epitopes recognized by H-2 restricted cytotoxic T lymphocytes specific for HLA using recombinant genes and synthetic peptides. *J. Immunol.* 140:871.
8. Romero, P., G. Corradin, I.F. Leuscher, and J.L. Maryanski. 1991. H-2K^d-restricted antigenic peptides share a simple binding motif. *J. Exp. Med.* 174:603.
9. MacDonald, H.R., N. Thiernesse, and J.-C. Cerottini. 1981. Inhibition of T cell-mediated cytolysis by monoclonal antibodies directed against Lyt2: heterogeneity of inhibition at the clonal level. *J. Immunol.* 126:1671.
10. Necker, A., N. Rebaï, M. Matthes, E. Jouvin-Marche, P.-A. Cazenave, P. Swarnworawong, E. Palmer, H.R. MacDonald, and B. Malissen. 1991. Monoclonal antibodies raised against engineered soluble mouse T cell receptors and specific for Vα8-, Vβ2- or Vβ10-bearing T cells. *Eur. J. Immunol.* 21:3035.
11. Frischauf, A.M., H. Lehrach, A. Ponstkas, and N. Murray. 1983. Lambda replacement vectors carrying polylinker sequences. *J. Mol. Biol.* 170:827.
12. Malissen, M., J. Trucy, F. Letourneur, N. Rebaï, D.E. Dunn, F.W. Fitch, L.E. Hood, and B. Malissen. 1988. A T cell clone expresses two T cell receptor α genes but uses one $\alpha\beta$ heterodimer for allorecognition and self MHC-restricted antigen recognition. *Cell.* 55:49.
13. Malissen, M., C. McCoy, D. Blanc, J. Trucy, C. Devaux, A.-M. Schmitt-Verhulst, F. Fitch, L.E. Hood, and B. Malissen. 1986. Direct evidence for chromosomal inversion during T cell receptor β gene rearrangements. *Nature (Lond.).* 319:28.
14. Casanova, J.-L., C. Pannetier, C. Jaulin, and P. Kourilsky. 1990. Optimal conditions for directly sequencing double-stranded PCR products with Sequenase. *Nucleic. Acids Res.* 18:4028.
15. Casanova, J.-L. 1992. Sequencing double-stranded linear DNA with Sequenase and ^{35}SαdATP. *In* Methods in Molecular Biology: DNA Sequencing. H. Griffin, editor. Humana Press, Inc., Clifton, NJ. In press.
16. Behlke, M.A., D.G. Spinella, H.S. Chou, W. Sha, D.L. Hartl, and D.Y. Loh. 1985. T cell receptor β chain expression: dependence on relatively few variable region genes. *Science (Wash. DC).* 229:556.
17. Gascoigne, N.R.J., Y.-H. Chien, D.M. Becker, J. Kavaler, and M.M. Davis. 1984. Genomic organization and sequence of T cell receptor β chain constant and joining region genes. *Nature (Lond.).* 310:387.
18. Becker, D.M., P. Patten, Y.-H. Chien, T. Yokota, Z. Eshhar, M. Giedlin, N.R.J. Gascoigne, C. Goodnow, R. Wolf, K.-I. Arai, and M.M. Davis. 1985. Variability and repertoire size of T cell receptor Vα gene segments. *Nature (Lond.).* 317:430.
19. Saito, H., D.M. Kranz, Y. Takagaki, A.C. Hayday, H. Eisen, and S. Tonegawa. 1984. A third rearranged and expressed gene in a clone of cytotoxic T lymphocytes. *Nature (Lond.).* 312:36.
20. Winoto, A., S. Mjolsness, and L.E. Hood. 1985. Genomic organization of the genes encoding mouse T cell receptor α chain. *Nature (Lond.).* 316:832.
21. Arden, B., J.L. Klotz, G. Siu, and L.E. Hood. 1985. Diversity and structure of genes of the α family of mouse T cell antigen receptor. *Nature (Lond.).* 316:783.
22. Chothia, C., D.R. Boswell, and A.M. Lesk. 1988. The outline structure of T cell $\alpha\beta$ receptor. *EMBO (Eur. Mol. Biol. Organ.) J.* 7:3745.
23. Brunner, K.T., and J.-C. Cerottini. 1971. Cytotoxic lymphocytes as effector cells of cell-mediated immunity. Symposium on effector mechanisms of cell-mediated immunity. *In* Progress in Immunology. B. Amos, editor. Academic Press, Inc., Orlando. 385–398.
24. Boon, T., J. Van Snick, A. Van Pel, C. Uyttenhove, and M. Marchand. 1980. Immunogenic variants obtained by mutagenesis of mouse mastocytoma P815. II. T lymphocyte-mediated cytolysis. *J. Exp. Med.* 152:1184.
25. Aebisher, T., S. Oehen, and H. Hengartner. 1990. Preferential usage of Vα4 and Vβ10 T cell receptor genes by lymphocytic choriomeningitis virus glycoprotein-specific H-2D^b restricted cytotoxic T cells. *Eur. J. Immunol.* 20:523.
26. Yanagi, Y., P. Macekawa, T. Cook, O. Kanagawa, and M.B.A. Oldstone. 1990. Restricted V-segment usage in T-cell receptors from cytotoxic T lymphocytes specific for a major epitope of lymphocytic choriomeningitis virus. *J. Virol.* 64:5919.
27. Romero, P., G. Eberl, J.-L. Casanova, A.-S. Cordey, C. Widmann, I.F. Luescher, G. Corradin, and J.L. Maryanski. 1992. Immunization with synthetic peptides containing a defined malaria epitope induces a highly diverse CTL response. Evidence that two peptide residues are buried in the MHC molecule. *J. Immunol.* 148:1871.
28. Kahn-Perles, B., C. Barra, J. Jehan, D. Fourel, M. Bard, J.L. Maryanski, and F. Lemonnier. 1989. Cytotoxic T lymphocyte recognition of secreted HLA class I molecules. *J. Immunol.* 142:3021.
29. Barra, C., B. Pérarnau, P. Gerlinger, M. Lemeur, A. Gillet, P. Gibier, and F. Lemonnier. 1989. Analysis of the HLA-Cw3-specific cytotoxic T lymphocyte response of HLA-B7 × human β2m double transgenic mice. *J. Immunol.* 143:3117.
30. Letourneur, F., and B. Malissen. 1989. Derivation of a T cell hybridoma variant deprived of functional T cell receptor α and β chain transcripts reveals a nonfunctional α-mRNA of BW5147 origin. *Eur. J. Immunol.* 19:2269.
31. Couez, D., M. Malissen, M. Buserne, A.-M. Schmitt-Verhulst, and B. Malissen. 1991. Each of the two productive T cell receptor α gene rearrangements found in both A10 and BM3.3 T cell clones gives rise to an α chain which can contribute to the constitution of a surface expressed $\alpha\beta$ dimer. *Int. Immunol.* 3:719.
32. Chou, H.S., M.A. Behlke, S.A. Godambe, J.H. Russel, C.G.

Brooks, and D.Y. Loh. 1986. T cell receptor genes in an alloreactive CTL clone: implications for rearrangement and germline diversity of variable gene segments. *EMBO (Eur. Mol. Biol. Organ.) J.* 5:2149.
33. Jouvin-Marche, E., I. Hue, P.N. Marche, C. Liebe-Gris, J.-P. Marolleau, B. Malissen, P.-A. Cazenave, and M. Malissen. 1990. Genomic organization of the mouse T cell receptor Vα family. *EMBO (Eur. Mol. Biol. Organ.) J.* 9:2141.
34. Winoto, A., J.L. Urban, N.C. Lan, J. Goverman, L.E. Hood, and D. Hansburg. 1986. Predominant use of a Vα gene segment in mouse T-cell receptors for cytochrome c. *Nature (Wash. DC).* 324:679.
35. Acha-Orbea, H., D.J. Mitchell, L. Timmerman, D.C. Wraith, G.S. Tausch, M.K. Waldor, S.S. Zamvil, H.O. McDevitt, and L. Steinman. 1988.Limited heterogeneity of T cell receptors from lymphocytes mediating autoimmune encephalomyelitis allows specific immune intervention. *Cell.* 54:263.
36. Tan, K.N., B.M. Datlof, J.A. Gilmore, A.C. Kronan, J.H. Lee, A.M. Maxam, and A. Rao. 1988. The T cell receptor Vα3 gene segment is associated with reactivity to p-azobenzenearsonate. *Cell.* 54:247.
37. Iwamoto, A., P.S. Ohashi, H. Pircher, C.L. Walker, E.E. Michalopoulos, F. Rupp, H. Hengartner, and T.W. Mak. 1987. T cell receptor variable gene usage in a specific cytotoxic T cell response. *J. Exp. Med.* 165:591.
38. Malissen, M., K. Minard, S. Mjolsness, M. Kronenberg, J. Goverman, T. Hunkapiller, M.B. Prystowsky, F. Fitch, T.W. Mak, and L.E. Hood. 1984. Mouse T cell antigen receptor: structure and organization of constant and joining gene segments encoding the β polypeptide. *Cell.* 37:1101.
39. Siu, G., M. Kronenberg, E. Strauss, R. Haars, T.W. Mak, and L.E. Hood. 1984. The structure, rearrangement and expression of Dβ gene segments of the murine T cell antigen receptor. *Nature (Lond.).* 311:344.
40. Kavaler, J., M.M. Davis, and Y.-H. Chien. 1984. Localization of a T cell receptor diversity element. *Nature (Lond.).* 310:421.
41. Hue, I., J. Trucy, C. McCoy, D. Couez, B. Malissen, and M. Malissen. 1990. A novel type of aberrant T cell receptor α chain gene rearrangement. Implications for allelic exclusion and the V-J recombination process. *J. Immunol.* 144:4410.