T Cell Receptor Genes in a Series of Class I Major Histocompatibility Complex–restricted Cytotoxic T Lymphocyte Clones Specific for a *Plasmodium berghei* Nonapeptide: Implications for T Cell Allelic Exclusion and Antigen-specific Repertoire

By Jean-Laurent Casanova,* Pedro Romero,‡ Christian Widmann,§ Philippe Kourilsky,* and Janet L. Maryanski‡

*From the *Biologie Moléculaire du Gène, INSERM U277, Institut Pasteur, 75015 Paris, France; the ‡Ludwig Institute for Cancer Research, Lausanne Branch, and the §Institut de Biochimie, Université de Lausanne, 1066 Epalinges, Switzerland*

Summary

We report here the first extensive study of a T cell repertoire for a class I major histocompatibility complex (MHC)-restricted cytotoxic T lymphocyte (CTL) response. We have found that the T cell receptors (TCRs) carried by 28 H-2K^d-restricted CTL clones specific for a single *Plasmodium berghei* circumsporozoite nonapeptide are highly diverse in terms of Vα, Jα, and Jβ segments and aminoacid composition of the junctional regions. However, despite this extensive diversity, a high proportion of the TCRs contain the same Vβ segment. These results are in contrast to most previously reported T cell responses towards class II MHC-peptide complexes, where the TCR repertoires appeared to be much more limited. In our study, the finding of a dominant Vβ in the midst of otherwise highly diverse TCRs suggests the importance of the Vβ segment in shaping the T cell repertoire specific for a given MHC-peptide complex. As an additional finding, we observed that nearly all clones have rearranged both TCR α loci. Moreover, as many as one-third of the CTL clones that we analyzed apparently display two productive α rearrangements. This argues against a regulated model of sequential recombination at the α locus and consequently raises the question of whether allelic exclusion of the TCR α chain is achieved at all.

The mouse TCR α/β is a disulphide-linked heterodimeric integral membrane glycoprotein. Each chain of ~40–45 kD contains a C and a V extracellular domain (1). The diversity of each α and β V domain results from the somatic recombination of ~100 Vα with ~50 joining α (Jα) gene segments and of ~20 Vβ with two diversity β (Dβ) and 12 joining β (Jβ) gene segments, respectively. Imprecise joining and addition of template-independent N-nucleotides further contribute to this diversity (2). The TCR is closely related to Ig by similar domain organization, overall sequence homology, and conservation of key residues. Along this line, Chothia et al. (3) have proposed an outline of the TCR tertiary structure, based on the known three-dimensional structure of Igs.

Whereas B cells recognize epitopes on native antigenic proteins, T cells can only recognize antigens in the context of cell surface syngeneic MHC molecules (4). The antigens recognized by T cells can be mimicked by synthetic peptides (5). The crystallographic structure of two class I MHC molecules has revealed a groove in the external domain, where the antigenic peptide could lie (6–8). There is now evidence that the antigens are naturally processed into short peptides that are loaded onto MHC molecules and exported at the cell surface (9–11).

The T cell specificity for an MHC-peptide complex is determined exclusively by the TCR (12). Accordingly, one question has received much attention. What is the diversity of the TCRs carried by T cells of a given specificity?

The determination of the primary structure of a number of TCRs carried by T cell clones or hybridomas of a given specificity, mostly MHC class II restricted, has been performed for a variety of protein antigens, either of eucaryotic origin, such as pigeon cytochrome *c* (pcc)[1] (13–19), beef insulin (Bi)

[1] *Abbreviations used in this paper:* Bi, beef insulin; CS, circumsporozoite; HA, hemagglutinin; HEL, hen egg lysozyme; LCMVgp, lymphochoriomeningitis virus glycoprotein; MBP, myelin basic protein; pcc, pigeon cytochrome *c*; SpWMb, sperm whale myoglobin.

 J. Exp. Med. © The Rockefeller University Press • 0022-1007/91/12/1371/13 $2.00
Volume 174 December 1991 1371–1383

(20, 21), hen egg lysozyme (HEL) (22), sperm whale myoglobin (SpWMb) (23), and murine myelin basic protein (MBP) (24, 25), or of viral origin, such as λ repressor cI (λcI) (26, 27), influenza virus hemagglutinin (HA) (28), and lymphochoriomeningitis virus glycoprotein (LCMVgp) (29, 30). Studies on hapten-specific T cells, for which the presented form of antigen is unknown, have been also undertaken (31–34).

The general picture that emerges from these studies is that T cells of a given class II MHC-peptide complex specificity bear TCRs that exhibit a limited diversity. This limitation concerns in most cases both α and β chains, and for each chain, both the V and J segments, and the amino acid composition of the junctional region, where for the β chain a conserved amino acid was observed. Strikingly, the selective pressure is such that T cells with identical TCR α and/or β chains have been isolated from different individual mice at a high frequency.

We have previously reported the isolation of H-2K^d-restricted CD8$^+$ CTL clones specific for the *P. berghei* circumsporozoite (CS) protein. (35, 36; Romero et al., manuscript in preparation). Notably, some of these clones were shown to protect the mice from malaria upon adoptive transfer. In this system, the nonapeptide PbCS 252-260 was shown to be the most potent peptide both in terms of K^d binding and CTL recognition (37).

We have now determined the primary structure of the α/β TCRs carried by 28 independent H-2K^d-restricted CTL clones specific for the *P. berghei* CS nonapeptide using cDNA-PCR followed by sequencing. To this end, we have developed and followed two original procedures. First, we have screened each T cell clone by PCR with a collection of 19 Vα and 20 Vβ oligonucleotides in conjunction with a Cα and a Cβ oligonucleotide, respectively. Second, we have set up new conditions for directly sequencing double-stranded PCR products (38).

In this first extensive analysis of a class I MHC-restricted response, we found that although the TCRs were highly diverse in terms of Vα, Jα, and Jβ segments and amino acid composition of the junctional regions, the response was paradoxically dominated by a Vβ segment. We also observed that nearly all clones had rearranged both TCR α loci and that in as many as one third of these clones, both rearrangements were apparently productive.

Materials and Methods

Cells. The isolation of CTL clones CS.B28, CS.B83, CS.C1, CS.C7, and CS.C11 from BALB/c mice immunized with *P. berghei* sporozoites has been described elsewhere (35). The other CTL clones analyzed in the present report were derived from mice immunized with synthetic *P. berghei* CS peptides. CTL clones that were originated from the same mouse are designated by a common capital letter followed by a number for each clone. The panel of 28 independent CTL clones was obtained from a total of 13 immune mice. Briefly, the CTL clones can be grouped into six categories based upon the strain of mice, the antigen used for in vivo priming, and the antigen used for in vitro stimulation as follows: (*a*) BALB/c mice immunized by intravenous injection of irradiated *P. berghei* sporozoites and their spleen cells stimulated in vitro with the PbCS 249-260 12-mer peptide (CTL B and C; total of five clones); (*b*) BALB/c mice immunized subcutaneously with the 10-mer PbCS 251-260 coupled to a helper epitope and their lymph node cells stimulated in vitro with the 10-mer peptide (Widmann et al., manuscript in preparation; CTL P, Q, and R; total of six clones); (*c*) (BALB/c × C57BL/6)F$_1$ mice immunized subcutaneously with the 12-mer PbCS 249-260 and their lymph node cells stimulated in vitro with the same peptide (CTL E, F, and M; total of seven clones); (*d*) (BALB/c × C57BL/6)F$_1$ mice immunized intravenously with a lipid derivative of the 9-mer PbCS 252-260 and their spleen cells stimulated in vitro with the 12-mer PbCS 249-260 (CTL H and L; total of four clones). (*e*) BALB/c mice immunized intravenously with the above lipopeptide and their spleen cells stimulated in vitro with the 12-mer PbCS 249-260 (CTL K; one clone). (*f*) (BALB/c × C57BL/6)F$_1$ mice immunized subcutaneously with the 8-mer PbCS 253-260 and their lymph node cells stimulated in vitro with the same peptide (CTL I and J; total of five clones). The details of in vivo priming and in vitro stimulation and cloning are reported elsewhere (35, and Romero et al., manuscript in preparation). Cloned cells were harvested 5–7 d after the last restimulation for cell surface staining or for RNA extraction.

Cell Surface Labeling with Vβ-specific mAbs. CTL clones were stained with several anti-Vβ mAbs: F23.1 (anti-Vβ8.1, 8.2, 8.3), F23.2 (anti-Vβ8.2) (39), KJ16 (anti-Vβ8.1, 8.2) (40), 44-22-1 (anti-Vβ6) (41), Mm VB-TCR-6B (anti-Vβ13) (PharMingen, San Diego, CA), 14-2 (anti-Vβ14) (42) and TR310 (anti-Vβ7) (43). Briefly, cells were stained with saturating amounts of the anti-Vβ mAb followed by an appropriate FITC-labeled anti-Ig second reagent or Avidin-PE. Samples were passed on a FACS II® flow cytometer (Becton Dickinson & Co., Sunnyvale, CA).

RNA Extraction, cDNA Synthesis, and PCR. Total RNA was extracted from 10^6 cells by disruption of the cells in guanidium thiocyanate followed by ultracentrifugation through a cesium chloride cushion. Pelleted RNA was thereafter extracted once with phenol-chloroform, ethanol precipitated, and suspended in 50 μl water. Single-stranded cDNA synthesis was carried out on 5 μg total RNA with oligo(dT)$_{15}$ and AMV reverse transcriptase (Boehringer Mannheim Biochemicals, Indianapolis, IN) according to manufacturer's instructions. After an ethanol precipitation, the cDNA was suspended in 100 μl water. PCR was carried out in 100 μl on 1/100 of the cDNA with 1.5 U of Taq polymerase (Cetus Corp., Emeryville, CA) according to manufacturer's instructions. The primers are listed in Table 1. 30 cycles, each of 95°C for 1 min, 55°C for 1 min, and 72°C for 1 min, were completed in a thermostater (Cetus Corp.).

Direct Sequencing of Double-stranded Linear DNA. The PCR products were ethanol precipitated and separated by electrophoresis on a 2% agarose gel. The band of interest was cut out, submitted to electroelution, and the eluted DNA ethanol precipitated. This double-stranded linear DNA was directly sequenced with the Sequenase version 2.0 kit (United States Biochemicals, Cleveland, OH) and ^{35}SdATP as described (38). Briefly, 0.4 pmol of template was boiled for 10 min in 10 μl with a 20-fold molar excess of sequencing primer. The sample was rapidly transferred to a dry-ice ethanol bath. Labeling mix (5.5 μl) was added to the frozen pellet and the tube allowed to warm. Once the ice melted, the solution was incubated for ~30 s at room temperature and then four 3.5-μl samples were transferred to the borders of wells containings 2.5 μl of the respective ddNTPs mixtures. The 96-well plaque was then spun down and incubated at 37°C for 2 min. The reaction was stopped by 5 μl of stop solution. The sequence products were separated on an 8 M urea, 6% acrylamide gel.

Probabilities. Let us consider that a rearrangement has a proba-

bility p_1 to be productive: p_1 depends on both the frequency of germline V, (D), and J pseudogenes and the frequency of unproductive V(D)J recombinations. Let us also consider a probability p_2 for a productive rearrangement to shut off the recombination of the other locus: p_2 depends on hypothetical post translational requirements, e.g., level of expression, heterodimer formation, cell surface expression, positive selection, etc. In any case, if the two α loci rearrange sequentially and if the recombination is regulated, the proportion of cells with one productive plus one unrearranged (α^+/α^0), two productive (α^+/α^+), and one productive plus one unproductive (α^+/α^-) α recombinations would be: $1/(2 - p_1p_2)$, $(1 - p_2)p_1/(2 - p_1p_2)$, and $(1 - p_1)/(2 - p_1p_2)$, respectively. Thus, since p_1 must be a value between 0 and 1/3, and p_2 between 0 and 1, these proportions can be estimated to be between 1/2 and 3/5, 1/6 and 0, and 1/3 and 2/5, respectively. Our observed values for any of the three categories differ significantly from these expected values. Even if we cannot rigorously discriminate between productive and unproductive rearrangements because we do not have the full-length sequences, it is established that only 4 of 28 CTL clones, at most, have still one unrearranged α locus, instead of 14–17 as expected. Consequently, a regulated model of sequential recombination is unlikely to hold at the TCR α loci.

Results

A collection of 47 CTL clones, H-2K^d restricted and specific for the PbCS nonapeptide 252-260, was derived from 13 mice and is described elsewhere (35, 36; Romero et al., manuscript in preparation). The combination of different mice and Vβ cell surface expression, as determined by fluorescence staining (see Materials and Methods), indicated that at least 23 clones were independent. All CTL clones were then tested by cDNA-PCR with a sense oligonucleotide specific for the Vβ determined by FACS® analysis and an antisense Cβ oligonucleotide (Table 1), followed by direct sequencing of the

Table 1. *Oligonucleotides*

Vβ*	Sequence (5′ to 3′)	Vα‡	Sequence (5′ to 3′)
Vβ1	CCCAGTCGTTTTATACCTGAATGC	Vα1	GCACTGATGTCCATCTTCTC
Vβ2	TCACTGATACGGAGCTGAGGC	Vα2	AAAGGGAGAAAAAGCTCTCC
Vβ3	CCTTGCAGCCTAGAAATTCAGTCC	Vα3	AAGTACTATTCCGGAGACCC
Vβ4	GCCTCAAGTCGCTTCCAACCTC	Vα4	CAGTATCCCGGAGAAGGTC
Vβ5.1	GTCCAACAGTTTGATGACTATCAC	Vα5	CAAGAAAGACAAACGACTCTC
Vβ5.2	AAGGTGGAGAGAGACAAAGGATTC	Vα6	ATGGCTTTCCTGGCTATTGCC
Vβ6	CTCTCACTGTGACATCTGCC	Vα7	TCTGTAGTCTTCCAGAAATC
Vβ7	TACAGGGTCTCACGGAAGAAGC	Vα8	CAACAAGAGGACCGAGCACC
Vβ8.1	CATTCTGGAGTTGGCTTCCC	Vα9	TAGTGACTGTGGTGGATGTC
Vβ8.2	CCTCATTCTGGAGTTGGCTACCC	Vα10	AACGTCGCAGCTCTTTGCAC
Vβ8.3	ACGCAAGAAGACTTCTTCCTCCTGC	Vα11	CCCTGCACATCAGGGATGCC
Vβ9	TCTCTCTACATTGGCTCTGCAGGC	Vα12	TCTGTTTATCTCTGCTGACC
Vβ10	ATCAAGTCTGTAGAGCCGGAGGAC	Vα13	ND
Vβ11	GCACTCAACTCTGAAGATCCAGAGC	Vα13.1	ACCTGGAGAGAATCCTAAGC
Vβ12	GAAGATGGTGGGGCTTTCAAGGATC	Vα34S-281	TCCTGGTTGACCAAAAAGAC
Vβ13	AGGCCTAAAGGAACTAACTCCAC	VαA10	TGGTTTGAAGGACAGTGGGC
Vβ14	ACGACCAATTCATCCTAAGCAC	VαBWB	CATTCGCTCAAATGTGAACAG
Vβ15	CCCATCAGTCATCCCAACTTATCC	VαBMA	CAAATGAGAGAGAGAAGCGC
Vβ16	CACTCTGAAAATCCAACCCAC	VαBMB	GGAAAATGCAACAGTGGGTC
Vβ18	CAGCCGGCCAAACCTAACATTCTC	Vα5T	GACATGACTGGCTTCCTGAAGGCCTTGC
Cβ§	Sequence (5′ to 3′)	Cα‖	Sequence (5′ to 3′)
Cβa	CCAGAAGGTAGCAGAGACCC	Cαa	TGGCGTTGGTCTCTTTGAAG
Cβb	CTTGGGTGGAGTCACATTTCTC	Cαb	ACACAGCAGGTTCTGGGTTC

* Vβ primers are sense oligonucleotides designed according to each Vβ gene segment. The Vβ gene segment nomenclature follows that of references 51, 67, 68, and 69.

‡ Vα primers are consensus sense oligonucleotides designed according to the known members of each Vα subfamily. The Vα gene subfamily nomenclature follows that of references 2, 45, 75, and 76. Nucleotide sequence of the Vα13 subfamily (2) was not available. The Vα5T gene segment sequence and specific primer were kindly provided by P. Marche (Institut Pasteur, Paris, France).

§ The Cβ primers are consensus antisense oligonucleotides for the Cβ1 and Cβ2 genes (71, 72). Cβb is located 3′ to Cβa.

‖ The Cα primers are antisense oligonucleotides designed according to the single Cα gene (77, 83). Cαb is located 3′ to Cαa.

A

CTL clone	Vβ			Jβ
B28	Vβ7		...TGT GCT AGC AGT TCC CGC TAT GAA CAG TAC TTC GGT...	Jβ2.7
M1	Vβ14		...TGT GCC TGG GGG ACT GGG GGG TTT GCT GAG CAG TTC TTC GGA...	Jβ2.1
RA10.3.3	Vβ14		...TGT GCC TGG AGC AAG GGG GCG CGA GGT CAA AAC ACC TTG TAC TTT GGT...	Jβ2.4
B83	Vβ6		...TGT GCC AGC ACC CCC ACC GGG ACA AAC AAC CAG GCT CCG CTT TTT GGA...	Jβ1.5
C11	Vβ6		...TGT GCC AGC ATC CCG ACG GCA AAC ACC GGG CAG CTC TAC TTT GGT...	Jβ2.2
	Vβ10	*	...TGT GCC AGC AGG CTA TGA ACA GTA CTT CGG T...	Jβ2.7
F12	Vβ6		...TGT GCC AGC ATC GGG ACA GGG GGC ACC GGG CAG CTC TAC TTT GGT...	Jβ2.2
	Vβ12	*	...TGT GCC AGC AGT TTT GGG ACA ACA ACC AGC TCC GCT TTT TGG A...	Jβ1.5
C1	Vβ8.1		...TGT GCC AGC AGT GTG ACA GGG TCA AAC ACA GAA GTC TTC TTT GGT...	Jβ1.1
H3	Vβ8.1		...TGT GCC AGC AGT GAT TCA CAG GGC ACA GAA GTC TTC TTT GGT...	Jβ1.1
F1	Vβ8.1		...TGT GCC AGC AGT GAT GAG GGA GTG GGG GAA AAC ACC TTG TAC TTT GGT...	Jβ2.4
	Vβ5.2	*	...TGT GCC AGC TCT CTC CGG GAC AGG GGA ACA CCG GGC AGC TCT ACT TTG GT...	Jβ2.2
QA11.3.2	Vβ8.1		...TGT GCC AGC AGG CCG GGA CAA CCC TAT GAA CAG TAC TTC GGT...	Jβ2.7
I7	Vβ8.2		...TGT GCC AGC GGT GAT GGA AAC CAG GCT CCG CTT TTT GGA...	Jβ1.5
F8	Vβ8.2		...TGT GCC AGC GGT GGG ACT GGG GGG GCA AAC ACC GGG CAG CTC TAC TTT GGT...	Jβ2.2
	Vβ6	*	...TGT GCC AGC AGT CAC TAG GGG AGG CCA AGA CAC CCA GTA CTT TGG G...	Jβ2.5
E22	Vβ13		...TGT GCC AGC AGT CCT ACA GGG AAA TCA AAC ACA GAA GTC TTC TTT GGT...	Jβ1.1
QB7.3.2	Vβ13		...TGT GCC AGC AGT CCC CCC CAG GTT GCA AAC ACA GAA GTC TTC TTT GGT...	Jβ1.1
H2	Vβ13		...TGT GCC AGC AGT CCC ACA GGT AGA AAC ACA GAA GTC TTC TTT GGT...	Jβ1.1
K1	Vβ13		...TGT GCC AGC AGG AGG CAG GGT GGC ACA GAA GTC TTC TTT GGT...	Jβ1.1
F15	Vβ13		...TGT GCC AGC AGT CCT CCT CAG GGG AAC CAA GAC ACC CAG TAC TTT GGG...	Jβ2.5
PF2.10.1	Vβ13		...TGT GCC AGC AGT TTC CGG GGG GGG CAA GAC ACC CAG TAC TTT GGG...	Jβ2.5
H1	Vβ13		...TGT GCC AGC AGT TCA GCC AGG GGT GAC ACC CAG TAC TTT GGG...	Jβ2.5
J3	Vβ13		...TGT GCC AGC AGG GAC AGG GGG CGC ACC AAC GAA AGA TTA TTT TTC GGT...	Jβ1.4
J5	Vβ13		...TGT GCC AGC AGT TCC CCA CAG GGA TCC AAC GAA AGA TTA TTT TTC GGT...	Jβ1.4
M2	Vβ13		...TGT GCC AGC AGT TTG GGA CAG GGG GCT TTT AAC TAT GCT GAG CAG TTC TTC GGA...	Jβ2.1
J2	Vβ13		...TGT GCC AGC AGT TTC CGG GAC AGG GGT AAC TAT GCT GAG CAG TTC TTC GGA...	Jβ2.1
L4	Vβ13		...TGT GCC AGC AGC CGA CTG GGG GCT TCC TAT GAA CAG TAC TTC GGT...	Jβ2.7
PE5.1.1	Vβ13		...TGT GCC AGC AGT TTC CAA TAT GAA CAG TAC TTC GGT...	Jβ2.7
J4	Vβ13		...TGT GCC AGC AGC CGC CGG GAC AGG GAT CAG GCT CCG CTT TTT GGA...	Jβ1.5
	Vβ1	*	...TGT GCC AGC AGC CAC GGG GAT TCA AAA CAC CTT GTA CTT TGG T...	Jβ2.4
C7	Vβ13		...TGT GCC AGC AGT CCG GGA CAG GGG CTC ACC GGG CAG CTC TAC TTT GGT...	Jβ2.2
	Vβ16	*	...TGT GCC AGC AGC CTA CTG GGG ACC AAG ACA CCC AGT ACT TTG GG...	Jβ2.5
RF3.10.3	Vβ13		...TGT GCC AGC AGT TCC GCT AGT GCA GAA ACG CTG TAT TTT GGC...	Jβ2.3

B

CTL clone	Vα			Jα
B28	Vα8.F3.3		...TGT GCT CTG AGT GGA GGT TCA GCC TTA GGG AGG CTG CAT TTT GGA...	JαLB2
	Vα4.δ7R	*	...TGT GCT CTG GGT GCC CTG GAG GAA GCA ATG CAA AGC TAA CTT CGG G...	JαB28
M1	Vα8.F3.3		...TGT GCT CTG GGT ACT GGA GGC AAT AAT AAG CTG ACT TTT GGT...	Jα2B4
	Vα5.TA72		...TGC GCA GTC AAT ATG GCT ACT GGA GGC AAT AAT AAG CTG ACT TTT GGT...	Jα2B4
RA10.3.3	Vα8.F3.3		...TGT GCT CTG AGT GGG TCG AAT CAA GGA GGG TCT GCG AAG CTC ATC TTT GGG...	JαTA27
	Vα5.MS202	*	...TGT GCA GTC AGT GTA GTG GAG GCA GCA ATT ACA AAC TGA CAT TTG GG...	Jα3DT
B83	Vα8.F3.4		...TGT GCT TTG AAC GGA GGT TCA GCC TTA GGG AGG CTG CAT TTT GGA...	JαLB2
	Vα1.B83	*	...TGT GCA GTG AGG TGA GCT CGG GAT ACA ACA AAC TCA CTT TTG GA...	JαB732
C11	Vα8.F3.5		...TGT GCT CTG AGT GAA ACA GGA GGT GCA GAT AGA CTC ACC TTT GGG...	JαTA80
F12	Vα8.F3.3		...TGT GCT CTG AGT GAT CAA GGA GGT GCA GAT AGA CTC ACC TTT GGG...	JαTA80
	Vα5T.J3	*	...TGT GCA GCA AGT GAA CAG GAA ACT ACA AAT ACG TCT TTG GA...	JαTA19
C1	Vα4.PJR25	*	...TGT GCT CTG AGT CTC GTC ATC TTC TGG CAG CTG GCA ACT CAT CTT TGG A...	JαTA28
H3	Vα10.TA57		...TGT GCT TTG GGC CTG TTC GGT GAC AAC AGT AAG CTG ATT TGG GGC...	JαBDFLI
	VαBMA.H3		...TGT GGC ACT GGA GCT AAC ACT GGA AAG CTC ACG TTT GGA...	JαC5
F1	Vα7.δ2B4Exp		...TGC GCT CTC TCG GAT CCG TCT AAT TAC AAC GTG CTT TAC TTC GGA...	Jα14.4
	Vα2.F1	*	...TGT GCA GCA AGT GCC GGA ATA ACA ATA ACA GAA TCT TCT TTG GT...	JαMD-13
QA11.3.2	Vα4.A1132		...TGT GCT CTG GTG AAT TAC AAC GTG CTT TAC TTC GGA...	Jα14.4
	Vα8.F3.4	*	...TGT GCT TTG AGT CTA CCA GGG AGG CAG AGC TCT GAT ATT TGG A...	JαA10
I7	Vα5.MDA		...TGC GCA GTC AGT GCC TAT GCA AAC AAG ATG ATC TTT GGC...	JαI7
	Vα4.A1132	*	...TGT GCT CTG CCA ATA ATA ATG CAG GTG CCA AGC TCA CAT TCG GA...	JαTA37
F8	Vα6.TA1		...TGT ATC CTG AGA GCG GGT TAC CAG AAC TTC TAT TTT GGG...	JαTA65
	Vα1.11.3	*	...TGT GCA GTT TGA ATC AAG GAG GGT CTG CGA AGC TCA TCT TTG GG...	JαTA27
E22	Vα8.F3.4		...TGT GCT TTG GTA AAT TCT GGG ACT TAC CAG AGG TTT GGA...	JαTA39
	Vα4.δ7R		...TGT GCT CTG AGT GAC GGG GAA CAT GGG CTA CAA ACT TAC TTC GGG...	Jα14T
QB7.3.2	Vα4.3		...TGT GCT CTG AGT CAT GGG ACT TAC CAG AGG TTT GGA...	JαTA39
	Vα8.F3.2		...TGT GCT TTG AGT GGG GAC GAC TCG GGA TAC AAC AAA CTC ACT TTT GGA...	JαB732
H2	Vα2.TA19		...TGT GCA GCA AGT GCA AAT TCT GGG ACT TAC CAG AGG TTT GGA...	JαTA39
	Vα7.2		...TGT GCT CTC TGG GAG CTG GCC GGA GGT TCA GAC CTT AGG GAG AGC TGC AAT TTT GGA...	JαH2
K1	Vα3.pHDS58		...TGT GCT GTG AGC ATG AAT GAA TAC AGA GGT GCA GAT AGA CTC ACC TTT GGG...	JαTA80
	Vα4.3		...TGT GCT CTG GGT GAG GGA AGC AAT GCA AAG CTA ACC TTC GGG...	JαK1
F15	Vα3.810		...TGT GCG GGG ACA GGC AAT ACC GGA AAA CTC ATC TTT GGA...	JαTA57
	VαBMB.42H11*		...TGT GCT ATG AGA GAG GGA ATT ATG GGG GCA GTG GCA ACA AGC TCA TCT TTG GA...	JαTT11
PF2.10.1	Vα3.AR5		...TGT GCT CTG AGC ATA ACA GGC AAT ACT AGA AAA CTC ATC TTT GGG...	JαC7
	Vα4.F2101		...TGT GCT CTG GAC CAG ACA GGC TTT GCA AGT GCG CTG ACA TTT GGA...	JαpHDS58
H1	Vα4.3		...TGT GCC CGC GGC ACC AAT ACA GGC AAA TTA ACC TTT GGG...	JαTA61
	Vα4.3	*	...TGT GTT CTG GGT GGG GGG GAG CAG TGG CAA CAA GCT CAT CTT TGG A...	Jα520K
J3	Vα5T.J3		...TGT GCG GGT AAT CAA GGA GGG TCT GCG AAG CTC ATC TTT GGG...	JαTA27
	Vα10.1F8	*	...TGT GCT ATG GTC ACG CGT CCA AGC AGC AAG GCA CTG GGT CTA AGC TGT CAT TTG GG...	JαTA1
J5	Vα4.3		...TGT CTC TGG GAA CTG GGA ACT GGG TCT AAG CTG TCA TTT GGG...	JαTA1
M2	VαBMA.M2		...TGT GTC TCA AAT TCT GGG ACT TAC CAG AGG TTT GGA...	JαTA39
	Vα4.MD13		...CCA AAT AAC TAT GCC CAG GGA TTA CCA TTC GGT...	Jα112.2
J2	Vα2.Ra9		...TGT GCA GCG GGC ATA ACT TTT GGG...	JαTA31
L4	Vα4.3		...TGT GCT CTG GGT GCC CAG GGA GGC AGA GCT CTG ATA TTT GGA...	JαA10
PE5.1.1	Vα2.8I		...TGT GCA GCA AGA GGA GGA AAC TAC AAA CCT ACG TTT GGG...	JαT6
	Vα8.F3.3		...TGT GCT CTG AGT GAT CAA GGA GGT GCA GAT AGA CTC ACC TTT GGG...	JαTA80
J4	VαBMA.P14		...TGT GCT GAC AAT AAC AGA ATC TTC TTT GGC...	JαMD13
	Vα1.E1	*	...TGT GCA GCT AGT GAG CAA TGG GGG TGG ATA GCA ACT ATC AGT TGA TCT GGG GC...	Jα1-27
C7	Vα3.pHDS58		...TGT GCT GCG AGT TAT GGG GGC AGT GGC AAC AAG CTC ATC TTT GGA...	JαTT11
	Vα8.F3.3	*	...TGT GCT CTG AGA AGG CAA TAC TAG AAA ACT CAT CAA TGG G...	JαC7
RF3.10.3	Vα4.F3103		...TGT GCT CTG GGT GAT CGG TAT GGG GGC AGT GGC AAC AAG CTC ATC TTT GGA...	JαTT11
	Vα3.pHDS58	*	...TGT GCT GTG AGC CAA CAG GCA ATA CTA GAA AAC TCA TCT TTG GG...	JαC7

Figure 1. TCR α and β cDNA junctional nucleotide sequences. (*A*) TCR β cDNA junctional sequences. Out-of-frame sequences are indicated by an asterisk. Nomenclature for Vβ gene segments follows that of references 67–69 and 51. Sequences are in references 67 (Vβ1, Vβ6, Vβ7), 70 (Vβ10, Vβ12, Vβ13), 68 (Vβ14, Vβ16), and 51 (Vβ5.2, Vβ8.1, Vβ8.2). The Jβ sequences are from references 71 and 72. The Vβ and Jβ gene segment sequences are identical to the published ones. However, two Jβ genes differ from the original reports: there is a base insertion (*underlined*) in the Jβ1.5 segment (. . . AACCAGGCT-CCG . . .) and a base substitution (*underlined*) in the Jβ1.4 segment (. . . CTGTCTGTCCTG . . .). Each of these changes occurred in all respective CTL clones. The Dβ segments are underlined (73, 74). The Cβ1 and Cβ2 genes could not be discriminated on a sequence basis and probably follow the Jβ cluster to which the Vβ is rearranged (71, 72). (*B*) TCR α cDNA junctional sequences. Out-of-frame sequences are indicated by an asterisk. Nomenclature for the Vα gene subfamilies follows that of references 2, 45, 75, and 76. The Vα subfamily is separated from the Vα gene segment by a period. The Vα gene segments are named according to the original cell in which they were isolated, from references 77 (3.pHDS58), 45 (5.TA72, 10.TA57, 6.TA1, 2.TA19), 78 (5.MDA, 4.MD13, 1.E1), 14 (4.3), 46 (8.F3.2, 8.F3.3, 8.F3.4, 8F3.5), 79 (7.δ2B4Exp), 33 (3.810), 21 (BMB.42H11), 26 (2.8I), 80 (BMA.P14), 81 (4.δ7R), 24 (4.PJ-R25), 75 (7.2), 34 (3.AR5), 17 (10.1F8), 82 (2.Ra9), 60 (5.MS202), 23 (1.11.3), and this report (1.B83, 5T.J3, BMA.H3, 2.F1, 4.A1132, 4.F2101, BMA.M2, 4.F3103). The Jα gene segments are from references 83 (TT11), 77 (pHDS58), 78 (LB2, MD13, 2B4, C5), 45 (TA27, TA80, TA19, TA28, TA37, TA65, TA39, TA57, TA61, TA1, TA31), 84 (BDFLI), 14 (14.4), 32 (112.2), 33 (520K), 75 (3DT), 59 (A10), 85 (14T), 86 (T6), 28 (1-27), and this report (B28, B732, I7, H2, K1, C7). The Vα and Jα gene segment sequences are identical to the published ones. However, JαMD13 in clone J-4 differs by a silent base substitution (*underlined*) from the original JαMD13 (. . . TTTGGC . . .). These sequences are available from EMBL/GenBank/DDBJ under accession numbers X60837 to X60921.

double-stranded PCR product. This procedure allowed a rapid determination of putative sister clones, i.e., CTL clones isolated from the same mouse and displaying an identical TCR β gene sequence. Thus, 28 independent CTL clones could be identified unambiguously (Fig. 1 *A*). All studies described below were carried out on these 28 clones.

Few CTL Clones Express Two Distinct VDJC β Transcripts. For each clone, the nucleotidic sequence of the amplified cDNA revealed an open reading frame encoding TCR β chain key residues (Fig. 1 *A* and Fig. 2). To further document these findings, we performed cDNA PCR on each CTL clone with a collection of 20 oligonucleotides specific for each of the known functional Vβ gene segments of the Vβ^b haplotype (Table 1). All these primers were shown to amplify the respective Vβ gene segment under the experimental conditions used. 6 of 28 CTL clones were found to express two different VDJC β transcripts. However, only one transcript was productive in every case (Fig. 1 *A* and Table 2). Since most if not all Vβ gene segments are known (44), the combination of cell surface stainings and nucleotide sequences indicated that the transcripts encoding the functional β chains were unambiguously determined (Fig. 2).

Nearly All CTL Clones Express Two Distinct VJC α Transcripts. We analyzed the VJC α transcripts by cDNA-PCR followed by direct sequencing. Each CTL clone was tested separately with 19 consensus oligonucleotides designed according to the known members of 19 Vα subfamilies in conjunction with a Cα oligonucleotide (Table 1). In contrast to the β transcripts, 23 of 28 CTL clones were found to express two different VJC α transcripts (Fig. 1 *B*). One of the five CTL clones in which only one VJC α transcript was detected, C1, contained an out-of-frame transcript, and thus presumably expressed a second undetected in-frame transcript. Another CTL clone, J5, was found to express an out-of-frame VDJC δ transcript (Casanova et al., manuscript in preparation). The remaining three clones, C11, J2, and L4, could possibly retain one unrearranged α locus. However, given the large estimated number of Vα gene segments and pos-

CTL clone	Vβ	FW	CDR3	FW	Jβ
B28	7	CAS	S S R Y E Q	YFG	2.7
M1	14	CAW	G T G G F A E Q	FFG	2.1
RA10.3.3	14	CAW	S K G A R G Q N T L	YFG	2.4
B83	6	CAS	T P T G T N N Q A P	LFG	1.5
C11	6	CAS	I P T A N T G Q L	YFG	2.2
F12	6	CAS	I G T G G T G Q L	YFG	2.2
C1	8.1	CAS	S V T G S N T E V	FFG	1.1
H3	8.1	CAS	S D S Q G T E V	FFG	1.1
F1	8.1	CAS	S D E G V G E N T L	YFG	2.4
QA11.3.2	8.1	CAS	R P G Q P Y E Q	YFG	2.7
I7	8.2	CAS	G D G N Q A P	LFG	1.5
F8	8.2	CAS	G G T G G A N T G Q L	YFG	2.2
E22	13	CAS	S P T G K S N T E V	FFG	1.1
QB7.3.2	13	CAS	S P P Q V A N T E V	FFG	1.1
H2	13	CAS	S P T G R N T E V	FFG	1.1
K1	13	CAS	R R Q G G T E V	FFG	1.1
F15	13	CAS	S P P Q G N Q D T Q	YFG	2.5
PF2.10.1	13	CAS	S F R G G Q D T Q	YFG	2.5
H1	13	CAS	S S A R G D T Q	YFG	2.5
J3	13	CAS	R D R G R T N E R L	FFG	1.4
J5	13	CAS	S S P Q G S N E R L	FFG	1.4
M2	13	CAS	S L G Q G A F N Y A E Q	FFG	2.1
J2	13	CAS	S F R D R G N Y A E Q	FFG	2.1
L4	13	CAS	S R L G A S Y E Q	YFG	2.7
PE5.1.1	13	CAS	S F Q Y E Q	YFG	2.7
J4	13	CAS	S R R D R D Q A P	LFG	1.5
C7	13	CAS	S P G Q G L T G Q L	YFG	2.2
RF3.10.3	13	CAS	S S A S A E T L	YFG	2.3

Figure 2. TCR β chain junctional amino acid sequences. 28 CTL clones are listed on the vertical axis. For each clone, the TCR β transcript with both an open reading frame and the triplets coding for key residues at the VDJ junction (3) was considered to encode the functional TCR β chain. For all clones (except B28, not stained by the antiVβ7 mAb), the FACS® stainings with anti-Vβ mAbs were in strict agreement with the β transcript assignments. The deduced amino acid sequences (in single-letter amino acid code) of the junctional, hypervariable and putatively CDR3-like regions, according to Chothia et al. (3), are represented. The presumed Ig-like loop, designated CDR3 for convenience, is putatively supported by two framework branches (FW). The Cys residue is at position 92 in the β chain. The Vβ and Jβ segments are also reported (see Fig. 1 for references).

Table 2. *Status of the TCR α and β V(D)JC Transcripts*

Status*	CTL clones	
	α	β
+/−	17‡ (61%)	6 (22%)
+/+	8 (29%)	0
+/0	3§ (10%)	22 (78%)

* The symbols + and − indicate transcripts that exhibit or not, respectively, an open reading frame at the V(D)JC junction, and the symbol 0 indicates the absence of transcript detected by cDNA PCR.
‡ Clones M2 (+/+) and J5 (+/0) are included in this category because they express either a nonfunctional, although in-frame, α transcript, due to the absence of the triplet coding for the key Cys residue at position 90, or a δ transcript, respectively. Clone C1 (0/−) is also included because it bears an α/β TCR on the cell surface and must therefore express a productive α transcript in addition to the unproductive one detected (see Fig. 3 *A*).
§ The three clones C11, J2, and L4 might express an additional α transcript that would not hybridize to the PCR primers (see Fig. 3 *B*).

sibly subfamilies of unknown sequences, these clones might rather express an α transcript that did not hybridize to the oligonucleotides used.

Among the 23 CTL clones that expressed two α transcripts, nine expressed both α transcripts in frame at the VJC junction. In one of them, M2, the triplet coding for the key Cys residue at position 90 was deleted (3). Because of partial sequencing, we cannot rule out that a Vα pseudogene segment could render one of the two α transcripts unproductive in a number of these clones. However, the estimated frequency of Vα pseudogenes is low (45–47). The remaining 14 CTL clones expressed one transcript in-frame and one out-of-frame (Table 2). Thus, for 16 CTL clones that expressed either a second unproductive α transcript (including M2) or a δ transcript (J5), the transcript encoding the functional α chain could be assigned unambiguously (Fig. 3 and Table 3).

The Vβ13 Gene Segment–encoded Region Is Overrepresented. Six Vβ gene segments from five different subfamilies are represented (Fig. 2). Vβ13 is predominant, found in 16 CTL clones (57%). The frequency of Vβ13 clones is lower among those from mice immunized with irradiated *P. berghei* sporozoites (1/5). Among CTL clones from peptide-immunized animals, the frequency of Vβ13 usage is 74% (15/23). The Vβ13 frequency among $CD8^+$ lymphocytes in these strains of mice is between 3% and 5% (data not shown). Furthermore, the Vβ13 predominance is unlikely to be the result of an increased representation of Vβ13 among H-$2K^d$-restricted T cells, since only one Vβ13 usage was found in the analysis of 25 independent H-$2K^d$-restricted CTL clones specific for other peptides (data not shown).

The TCR β Chain Primary Structures Are Otherwise Highly Diverse. All 28 TCR β chains differed from each other, and apart from the Vβ13 usage, no other predominant structural features were identified for the β chain repertoire. Indeed, 10 Jβ segments, out of 12 possible genomic segments, are used among 28 CTL clones. Even among the 16 Vβ13 CTL clones, eight different Jβ segments are used. Likewise, no preferential Jβ segment usage was observed for the other Vβ segments. Furthermore, the length of the CDR3 loop, defined according to Chothia et al. (3), varies from 6 to 12 amino acids, without striking dominant intermediate values. The CDR3 lengths are also highly variable among CTL clones that share a given Vβ segment and even among those that share a given Vβ-Jβ pair. When all loops of a given length were compared for the amino acid composition at a given position, no conservation could be found. Even when positions were assigned with respect to the Cys 92 residue (3), loops with different lengths did not show any obvious amino acid conservation. Moreover, even loops of a given length and supported by a given Vβ or Jβ framework failed to show amino acid conservation in the non-Vβ- or non-Jβ-encoded regions, respectively.

The Functional TCR α Chains Are Also Highly Diverse. The 16 known functional TCR α chains were found to differ from each other (Fig. 3). No Vα subfamily predominance such as the Vβ 13 among Vβs was observed. Overall, 8 different Vα subfamilies, 13 different Vα gene segments, and 13 different Jα segments were found among the 16 CTL clones.

Table 3. *Gene Segments Used by Unambiguous Functional TCR Heterodimers*

CTL clone*	Vβ‡	Jβ	Vα	Jα
B28	7	2.7	8.F3.3	LB2
RA10.3.3	14	2.1	8.F3.3	TA27
B83	6	1.5	8.F3.4	LB2
F12	6	2.2	8.F3.3	TA80
F1	8.1	2.4	7.δ2B4Exp	14.4
QA11.3.2	8.1	2.7	4.A1132	14.4
I7	8.2	1.5	5.MDA	I7
F8	8.2	2.2	6.TA1	TA65
F15	13	2.5	3.810	TA57
H1	13	2.5	4.3	TA61
J3	13	1.4	5T.J3	TA27
J5	13	1.4	4.3	TA1
M2	13	2.1	BMA.M2	TA39
J4	13	1.5	BMA.P14	MD13
C7	13	2.2	3.pHDS58	TT11
RF3.10.3	13	2.3	4.F3103	TT11

* For 16 CTL clones, the functional α chain, engaged in heterodimeric formation with the β chain and specific of the H-$2K^d$-PbCS252-260 combination, was unambiguously determined. Indeed, 14 clones express a second, out-of-frame, α transcript. In addition, clone J5 expresses a δ transcript, and one of the two in-frame α transcripts from clone M2 has deleted the triplet coding for the key Cys residue at position 90.
‡ Nomenclature and references for the Vβ, Jβ, Vα, and Jα gene segments are in the legend to Fig. 1.

CTL	Vα	FW	CDR3	FW	Jα
A					
B28	8.F3.3	CAL	S G G S A L G R L	HFG	LB2
RA10.3.3	8.F3.3	CAL	S G S N Q G G S A K L	IFG	TA27
B83	8.F3.4	CAL	N G G S A L G R L	HFG	LB2
F12	8.F3.3	CAL	S D Q G G A D R L	TFG	TA80
C1					
F1	7.δ2B4Exp	CAL	S D P S N Y N V L	YFG	14.4
QA11.3.2	4.A1132	CAL	V N Y N V L	YFG	14.4
I7	5.MDA	CAV	S A Y A N K M	IFG	I7
F8	6.TA1	CIL	R A G Y Q N F	YFG	TA65
F15	3.810	CAG	T G N T G K L	IFG	TA57
H1	4.3	CAR	G T N T G K L	TFG	TA61
J3	5T.J3	CAG	N Q G G S A K L	IFG	TA27
J5	4.3	CLW	E L G T G S K L	SFG	TA1
M2	BMA.M-2	CVS	N S G T Y Q	RFG	TA39
J4	BMA.P14	CAD	N N R I	FFG	MD13
C7	3.pHDS58	CAA	S Y G G S G N K L	IFG	TT11
RF3.10.3	4.F3103	CAL	G D R Y G G S G N K L	IFG	TT11
B					
C11	8.F3.5	CAL	S E T G G A D R L	TFG	TA80
J2	2.Ra9	CAA	G I	TFG	TA31
L4	4.3	CAL	G A Q G G R A L	IFG	A10
C					
M1	8.F3.3	CAL	G T G G N N K L	TFG	2B4
	5.TA72	CAV	N M A T G G N N K L	TFG	2B4
H3	10.TA57	CAL	G L L G D N S K L	IWG	BDFLI
	BMA.H3	CGT	G A N T G K L	TFG	C5
E22	4.δ7R	CAL	S D G E H G L Q T	YFG	14T
	8.F3.4	CAL	V N S G T Y Q	RFG	TA39
QB7.3.2	4.3	CAL	S H G T Y Q	RFG	TA39
	8.F3.2	CAL	S G D D S G Y N K L	TFG	B732
H2	2.TA19	CAA	S A N S G T Y Q	RFG	TA39
	7.2	CAL	W E L A G G S D L R E S C	NFG	H2
K1	3.pHDS58	CAV	S M N E Y R G A D R L	TFG	TA80
	4.3	CAL	G E G S N A K L	TFG	K1
PF2.10.1	3.AR5	CAL	S I T G N T R K L	IFG	C7
	4.F2101	CAL	D Q T G F A S A L	TFG	pHDS58
PE5.1.1	2.8I	CAA	R G G N Y K P	TFG	T6
	8.F3.3	CAL	S D Q G G A D R L	TFG	TA80

Figure 3. TCR α chain junctional amino acid sequences. The 28 CTL clones are separated in three groups, as in Table 2. Group *A* (17 clones) gathers 14 clones for which the functional α chain can be unambiguously assessed due to the presence of a second, out-of-frame, α transcript. It also includes clone J5, which expresses a δ transcript, and clone M2, which expresses two in-frame α transcripts, one of them being, however, nonfunctional since it has deleted the triplet coding for the conserved Cys residue at position 90. Clone C1, for which we could not detect a productive α transcript, is also included. Group *B* (three clones) gathers clones for which only one α transcript was detected and found to be productive. Thus, we can not affirm that the corresponding α chain is necessarily functional. Group *C* (eight clones) gathers clones that express two α transcripts in frame at the VJ junction, both potentially encoding a functional α chain. For each group, the α chain–deduced amino acid sequence (in the single-letter code) of the CDR3-equivalent loop, according to Chothia et al. (3), is reported with the Vα and Jα segments (for references see Fig. 1). The key Cys residue is at position 90 in the α chain.

The CDR3 lengths were found to be extremely variable, ranging from 4 to 11 amino acids and without any particular distribution. Moreover, no obvious amino acid conservation was found, even when loops of a given length and/or supported by a given Vα or Jα framework were compared. When all different possible α chains from the CTL clones for which the functional α chain was only putative were included in the analysis, the structural diversity was further increased. Altogether, the TCR α chain primary structures were found to be highly diverse.

Discussion

We have analyzed the TCRs from 28 independent CTL clones specific for the PbCS nonapeptide 252-260 presented by the H-2K^d restriction element. The sequences of the amplified TCR α and β cDNAs were determined. From this analysis, two major observations were made. First, nearly all CTL clones have rearranged both TCR α loci, and as many as one third of these clones apparently display two productive α rearrangements. Second, although all TCR α and β chains differ from each other and are highly diverse in terms

of both Vα, Jα, Jβ segments and amino acid composition of the junctional regions, where no conserved amino acid was found, there is a strong dominance of the Vβ13 segment.

Rearrangements of the VDJ β Gene Segments. All CTL clones express only one productive VDJC β transcript and a few (22%) express an additional unproductive one (Table 2). This study at the mRNA level by cDNA-PCR provides reasonably reliable information on genomic VDJ rearrangements. Similar proportions at the genomic level are described for the Ig H chain locus in B cells, where a regulated model of sequential recombination is believed to account for allelic exclusion (48–50). In this model, a complete VDJ rearrangement occurs first on one chromosome, and only if this is not productive can the other locus then rearrange. Such a model predicts that on the average 40–50% of peripheral cells should contain two distinct VDJ rearrangements (see Materials and Methods). The somewhat lower level (22%) in the present study could be explained in part by rearrangements involving a Vβ pseudogene of the $V\beta^b$ haplotype (44, 51, 52), for which we did not design specific oligonucleotides. Since most if not all TCR Vβ gene segments are presumably described, rearrangements involving new Vβ segments are very unlikely (44). Surprisingly, two mouse T cell clones harboring two productive β rearrangements have recently been reported (53, 54). The relatively large series analyzed here suggests that the frequency of such cells is very low and that consequently they may have little biological significance. Altogether, the TCR β gene VDJ rearrangements appear to be consistent with a regulated model of sequential recombination that maintains a strict allelic exclusion for the TCR β chain.

Rearrangements of the VJ α Gene Segments. In contrast to our findings for the β transcripts, nearly all CTL clones analyzed express two distinct α VJC transcripts (Table 2). Few previous studies have analyzed the status of rearrangements at both α loci in T cell clones, mainly because of the size of the Jα locus, which extends over a stretch of 60 kb and thereby makes the analysis by Southern blot difficult and sometimes ambiguous (55–58). In one study, only 3 of 10 T cell clones were reported to be rearranged at both α loci (58). In contrast, Malissen and colleagues (55) reported that both α loci were rearranged in eight of nine T cell clones. Our cDNA-PCR approach with an extensive series of Vα primers followed by sequencing has allowed us to demonstrate unambiguously that at least 24 of 28 clones have rearranged both α loci. Thus, it is likely that both TCR α loci are generally rearranged in most peripheral α/β T cells.

At least 8 of the 28 CTL clones (29%) specific for the *P. berghei* CS nonapeptide appear to express two productive α transcripts. In the literature, three T cell clones have been described that clearly bear two productive α rearrangements (59–62), but in the absence of an extensive study to estimate the frequency of such cells, their biological relevance was unclear. Our study now indicates that the occurrence of T cells that express two productive α transcripts is probably rather frequent. Moreover, it raises the question of whether TCR α chain allelic exclusion (i.e., the presence of a single heterodimer at the cell surface) is actually achieved in such lymphocytes.

Altogether, these results on the TCR α gene VJ rearrangements strongly suggest that the recombination events at the TCR α loci differ considerably from those at the Ig and TCR β loci, and that a regulated model of sequential recombination may not hold for the TCR α genes (see Materials and Methods).

Diversity of the TCR α and β Chains. Several class II MHC-restricted helper T cell responses to well-defined peptides have been reported. For some antigens, the number of sequences is too low to give an idea of the actual repertoire. For those studies with a sufficient number of sequences, there is clearly the occurrence of not only a dominant Vβ segment but also an associated dominant Jβ segment, a common CDR3 length, and a conserved amino acid in the non-Vβ-, non-Jβ-encoded part of the CDR3 loop (Table 4). However, the diversity of the repertoire varies slightly from case to case and the response to HA appears to be the most diverse. In addition, a very striking feature is the fact that T cell clones bearing TCR β chains identical at the amino acid level could be isolated in all these studies from different individual mice at a high frequency. In contrast, apart from the Vβ13 dominance, we found no structural limitations in the CTL response to the *P. berghei* CS nonapeptide-K^d complex and no identical β chains, despite a larger number of clones analyzed.

A striking recurrence of particular combinations of Vα gene subfamily, Jα segment, and CDR3 length has been observed in most class II MHC-restricted T cell responses analyzed (Table 5). Most strikingly, T cell clones bearing identical α chains were isolated from different individual mice. This is again compelling evidence for a very strong selective pressure, given the potential diversity of the receptor chains. As an exception, the response to HA appears to be more diverse. However, the function of the proteins encoded by the α transcripts in the latter study is only putative, since data on the second TCR α locus are lacking. In the present study, in which we analyzed not only many but, most importantly, unambiguously assigned functional α chains, we found no dominant Vα usage, no limitations on the junctional structures, and no identical α chains.

For the 16 CTL clones in our study where the functional α/β pairing is unambiguous (Table 3), there is no obvious preferential Jα-Jβ pairing, nor Vα-Vβ pairing. Rather, there is a large diversity of pairing between chains encoded by different Vα and Vβ gene subfamilies or segments. Thus there are 11 or 15 different α/β pairs, considering V gene subfamilies or segments, respectively, out of 16 TCRs. Notably, the Vβ13 and Vβ8 subfamily-encoded chains appear to be mutually exclusive with those of Vα8. If the remaining 12 clones are included in the analysis, the diversity of pairing is further increased. In contrast, most other T cell responses analyzed to date display limited pairing diversity. Of these, the HA response appears to be the most diverse, but again, the α chains are only putative and so the pairing among functional chains could possibly be less diverse. In many studies, TCRs identical not only for one chain but for both the α and β chains were isolated from different individual mice (Table 5).

Each of the 28 CTL clones has a unique fine specificity pattern when tested for recognition of a series of Ala-

Table 4. *Comparison of the TCR β Chain Repertoires Reported for Various Antigens*

MHC-peptide complex*	Strain‡	Total TCR β§	Identical TCR β‖	Dominant Vβ¶ (*n*)	Dominant Jβ (*n*)	Dominant length (*n*)	Conserved amino acid**
MHC class II							
pcc_{81-104}/IE^k	B10.A, F_1/A	15	2,2,2,2,2	Vβ3 (7)	Jβ1.2 (5)	9 (5)	N
				Vβ16 (6)	Jβ2.1 (6)	9 (6)	DT
pcc_{81-104}/IE^s	B10.S(9R), F_1/9R	7	2	Vβ1 (6)	Jβ1.2 (5)	8 (3)	D
$SpWMb_{110-121}/IE^d$	DBA/2	6	3	Vβ8.2 (6)	Jβ2.7 (5)	10 (5)	WDW
MBP_{1-9NAc}/IA^u	PL/J, (P × S) F_1	8	2,2,2	Vβ8.2 (7)	Jβ2.7 (4)	8 (4)	GLG
	B10.PL	6	4	Vβ8.2 (5)	Jβ2.7 (5)	9 (5)	AGG
$\lambda cI_{12-26}/IE^k$	A/J	11	3,2	Vβ1 (8)	Jβ2.1 (7)	10 (7)	E
$HA_{110-120}/IE^d$	BALB/c	13	3	Vβ8.3 (7)	Jβ1.3 (4)	8 (4)	G
MHC class I							
$PbCS_{252-260}/K^d$	BALB/c, (B × C)F_1	28	NF‡‡	Vβ13 (16)	NF	NF	NF

* For references, see Introduction. Only studies with at least six sequenced TCRs specific for a given MHC-peptide complex are included. Thus, studies on HEL-, Bi-, pcc (B10S[5R])- and LCMV-specific T cells are not reported. Studies on hapten-specific T cells are not mentioned because the processed form of the antigen is unknown.

‡ Responses to a given MHC-peptide combination in different strains are considered separately, with the exceptions of chimeras (F_1/A [(B10A × B10S[9R])F_1 bone marrow in B10.A irradiated host] and F_1/9R [(B10.A × B10.S[9R])F_1 bone marrow in irradiated B10.S(9R)], as well as hybrids (P × S)F_1 ([PL.J × SJL]F_1) and (B × C)F_1 ([BALB/c × C57BL/6]F_1).

§ Only sequenced TCR β chains are considered. The numbers indicate the total number of sequenced β chains for each particular specificity and strain. Probable sister clones, i.e., clones displaying the same TCR gene sequence and coming from the same animal, are excluded.

‖ Only identical TCR β chains isolated from different individual mice are reported, irrespective of the Vβ or Jβ usage. Each number indicates how many chains share a given structure. For example 3,2 means that two distinct groups of identical chains were found, one with three members, and one with two members.

¶ Any element, gene segment (Vβ, Jβ) or CDR3 length (3), is said to be dominant if it represents more than a third of the previous element(s), in the order of the Table. The nature of these elements in each case is given in the Vβ, Jβ, and length columns, respectively. *n* indicates the number of TCRs sharing this element among the ones carrying the previous one(s).

** Conserved amino acid indicates the non-Vβ, non-Jβ-encoded conserved residue (single-letter code) in the CDR3 loop among TCRs sharing the previous dominant Vβ-Jβ-length combination (only N in the pcc [B10.A] response is not absolutely conserved, present in four out of five TCR β chains).

‡‡ None found.

Table 5. *Comparison of the TCR α Chain Repertoires Reported for Various Antigens*

MHC-peptide* complex	Strain‡	Total TCR α§	Identical TCR α‖	Identical TCRα/β	Dominant Vα (*n*)	Dominant Jα (*n*)	Dominant length (*n*)
MHC class II							
pcc_{81-104}/IE^k	B10.A, F_1/A	16	4,2,2,2	2,2,2	Vα11 (15)	JαTA84 (10)	9 (6)
$SpWMb_{110-121}/IE^d$	DBA/2	6	3	NF	Vα1 (4)	JαC5 (3)	10 (3)
MBP_{1-9NAc}/IA^u	PL/J, (P × S)F_1	8	3,2	2	Vα4 (8)	JαTA31 (6)	9 (6)
	B10.PL	7	6	3	Vα2 (6)	JαTA39 (6)	8 (6)
$\lambda cI_{12-26}/IE^k$	A/J	11	3,3	3	Vα2 (11)	JαTA1 (7)	8 (6)
$HA_{110-120}/IE^d$	BALB/c	13	NF	NF	NF	NF	NF
MHC class I							
$PbCS_{252-260}/K^d$	BALB/c, (B × C)F_1	16	NF	NF	NF	NF	NF

For abbreviations and references, see Table 4. The Vα indicates subfamilies. As opposed to the β chain, the non-V, non-J-encoded region of the α chain is too small and unprecise to look for amino acid conservation.

substituted related peptides (Romero et al., manuscript in preparation). The clones thus appear to recognize a large number of epitopes on the CS nonapeptide-K^d complex. This heterogeneity of fine specificity patterns clearly correlates with the diversity of TCR primary structure found for this set of CTL clones specific for the *P. berghei* CS nonapeptide-K^d complex.

A Paradoxical Vβ Dominance. As mentioned previously, the dominance of the Vβ13 gene segment may be peptide related, at least in part, since other K^d-restricted responses apparently fail to show a Vβ13 dominance. In agreement, a similar Vβ dominance has been reported for the other responses studied, with no apparent correlation to the restriction element (Table 4). In the currently prevailing models of TCR-MHC-peptide interaction (3, 63, 64), the CDR-1-like and CDR2-like loops of both α and β chains, encoded by the Vα and Vβ gene segments, respectively, are thought to interact with MHC residues, whereas the CDR3-like loops would interact with the peptide. This general topology was based on the much higher variability of the CDR3 loop and the peptide, when compared to CDR1 and CDR2 loops and the MHC molecule, respectively. It is now supported by increasing experimental evidence, showing that naturally occurring (14, 16, 18, 23, 27, 28) and experimentally engineered (65) TCRs that vary only in a CDR3 loop display distinct peptide fine specificities. Accordingly, an apparently peptide-related Vβ dominance in the context of otherwise highly diverse TCRs, such as we found in this study, is paradoxical. In the other studies, the overall diversity of the TCRs is very limited, thus, the Vβ dominance might be considered as an indirect consequence of a peptide-related constraint acting on other parts of the receptor, for example on the conserved amino acid in the CDR3 loop thought to be a peptide contact residue (16, 27, 65). In contrast, the high diversity of the TCRs in this study strongly suggests that the Vβ dominance in general might result from direct peptide-related constraints imposed by the peptide-MHC complex. Whether the Vβ dominance reflects a direct interaction of the Vβ segment with the peptide or an indirect effect of the bound peptide on the complex that in turn would favor the interaction of the Vβ segment with the restriction element is unknown.

What Determines the Size of the TCR Repertoire? Apart from the Vβ dominance, the extent of diversity of the T cell repertoire appears to vary considerably according to the MHC-peptide ligand involved. Why the CTL response to a *P. berghei* nonapeptide appears to be more diverse than the other reported T cell responses is unknown. It may be significant that the latter studies analyzed class II MHC-restricted T helper responses. This difference might allow a compensation for the apparently lower number of class I–than class II–restricted antigenic sites within proteins.

More likely, the size of the MHC-peptide complex–specific repertoire would depend primarily on the overlap between epitopes displayed by self peptides and the antigenic peptide bound to the same restriction element. This would explain why the responses towards polymorphic variants of self proteins (pcc, SpWMb) are so limited and the autoimmune response against MBP is oligoclonal. Even the λcI peptide, although of viral origin, has been shown by Gefter and colleagues (66) to be homologous to a self peptide able to bind the same restriction element. Conversely, responses to the viral HA determinant and the parasite CS peptide may be more diverse because relatively few self peptides would generate overlapping tolerogen epitopes. Although the CTL response to the whole *P. berghei* CS protein in H-2^d mice is focused primarily on a single nonapeptide in the context of H-$2K^d$, this peptide may be so distant to self that it triggers a highly diverse, and presumably highly potent, T cell response.

We thank J.-C. Cerottini for helpful discussions and his continuing interest and support. We are indebted to B. Malissen, M. Malissen, P. Marche, E. Jouvin-Marche, and A.-C. Garapin for helpful discussions and communication of unpublished results. We also acknowledge H. Acha-Orbea, G. Corradin, T. Crompton, G. Eberl, C. Jaulin, J. Kanellopoulos, T. Herrmann, H. R. MacDonald, B. Malissen, and C. Pannetier for helpful comments and critical reading of the manuscript, E. Mottez for oligonucleotides synthesis, and A.-S. Cordey, and K. Mühlethaler for superb technical assistance. J.-L. Casanova would like to express his gratitude to Steven E. Ross for his most helpful encouragement and support.

J.-L. Casanova was supported in part by the Institut National de la Santé et de la Recherche Médicale and in part by the European Organization for the Research on Treatment of Cancer. P. Romero was supported by the Roche Research Foundation.

Address correspondence to J.-L. Casanova, Ludwig Institute for Cancer Research, Lausanne Branch, Chemin des Boveresses 155, 1066 Epalinges, Switzerland.

Received for publication 17 June 1991 and in revised form 12 August 1991.

References

1. Klausner, R.D., J. Lippincott-Schwartz, and J.S. Bonifacino. 1990. The T cell antigen receptor: insights into organelle biology. *Annu. Rev. Cell Biol.* 6:403.
2. 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.
3. Chothia, C., D.R. Boswell, and A.M. Lesk. 1988. The outline structure of T cell αβ receptor. *EMBO (Eur. Mol. Biol. Organ.) J.* 7:3745.

4. Schwartz, R. 1985. T lymphocyte recognition of antigen in association with gene products of the major histocompatibility complex. *Annu. Rev. Immunol.* 3:237.
5. Milich, D.R. 1989. Synthetic T and B cell recognition sites: implications for vaccine development. *Adv. Immunol.* 45:195.
6. Bjorkmann, P.J., M.A. Saper, B. Samraoui, W.S. Bennett, J.L. Strominger, and D.C. Wiley. 1987. Structure of the human class I histocompatibility antigen, HLA-A2. *Nature (Lond.).* 329:506.
7. Bjorkman, P.J., M.A. Saper, B. Samraoui, W.S. Bennett, J.L. Strominger, and D.C. Wiley. 1987. The foreign antigen binding site and T cell recognition regions of class histocompatibility antigens. *Nature (Lond.).* 329:512.
8. Garrett, T.P.J., M.A. Saper, P.J. Bjorkmann, J.L. Strominger, and D.C. Wiley. 1989. Specificity pockets for the side chains of peptide antigen in HLA-Aw68. *Nature (Lond.).* 342:692.
9. Demotz, S., H.M. Grey, E. Appella, and A. Sette. 1989. Characterization of a naturally processed MHC class II restricted T cell determinant of hen egg lysozyme. *Nature (Lond.).* 342:682.
10. Van Bleek, G.M., and S.G. Nathenson. 1990. Isolation of an endogenously processed immunodominant viral peptide from the class I H-2K^b molecule. *Nature (Lond.).* 348: 213.
11. Rotzschke, O., K. Falk, K. Deres, H. Schild, M. Norda, J. Metzger, G. Jung, and H.-G. Rammensee. 1990. Isolation and analysis of naturally processed viral peptides as recognized by cytotoxic T cells. *Nature (Lond.).* 248:252.
12. Matis, L.A. 1990. The molecular basis of T cell specificity. *Annu. Rev. Immunol.* 8:65.
13. Fink, P.J., L.A. Matis, D.L. McElligott, M. Bookman, and S.M. Hedrick. 1986. Correlations between T-cell specificity and the structure of the antigen receptor. *Nature (Lond.).* 321:219.
14. 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 (Lond.).* 324:679.
15. Sorger, S.B., S.M. Hedrick, P.J. Fink, M.A. Bookman, and L.A. Matis. 1987. Generation of diversity in T cell receptor repertoire specific for pigeon cytochrome c. *J. Exp. Med.* 165:279.
16. Hedrick, S.M., I. Engel, D.L. McElligott, P.J. Fink, M.-L. Hsu, D. Hansburg, and L.A. Matis. 1988. Selection of amino acid sequences in the beta chain of the T cell antigen receptor. *Science (Wash. DC).* 239:1541.
17. McElligott, D.L., S.B. Sorger, L.A. Matis, and S.M. Hedrick. 1988. Two distinct mechanisms account for the immune response (Ir) gene control of the T cell response to pigeon cytochrome c. *J. Immunol.* 140:4123.
18. Sorger, S.B., Y. Paterson, P.J. Fink, and S.M. Hedrick. 1990. T cell receptor junctional regions and the MHC molecule affect the recognition of antigenic peptides by T cell clones. *J. Immunol.* 144:1127.
19. Sorger, S.B., and S.M. Hedrick. 1990. Highly conserved T-cell receptor junctional regions. Evidence for selection at the protein and the DNA level. *Immunogenetics.* 31:118.
20. Spinella, D.G., T.D. Hansen, W.D. Walsh, M.A. Behlke, J.P. Tillinghast, H.S. Chou, P.J. Whitely, J.A. Kapp, C.W. Pierce, E.M. Shevach, and D.Y. Loh. 1987. Receptor diversity of insulin-specific T cell lines from C57BL (H-2^b) mice. *J. Immunol.* 138:3991.
21. Sherman, D.H., P.S. Hochman, R. Dick, R. Tizard, K.L. Ramachandran, R.A. Flavell, and B.T. Huber. 1987. Molecular analysis of antigen recognition by insulin-specific T-cell hybridomas from B6 Wild-type and bm 12 mutant mice. *Mol. Cell Biol.* 7:1865.
22. Johnson, N.A., F. Carland, P.M. Allen, and L.H. Glimcher. 1989. T cell receptor gene segment usage in a panel of hen-egg white lysozyme specific, I-A^k-restricted T helper hybridomas. *J. Immunol.* 142:3298.
23. Danska, J.S., A.M. Livingstone, V. Paragas, T. Ishihara, and C.G. Fathman. 1990. The presumptive CDR3 regions of both T cell receptor α and β chains determine T cell specificity for myoglobin peptides. *J. Exp. Med.* 172:27.
24. 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.
25. Urban, J.L., V. Kumar, D.H. Kono, C. Gomez, S.J. Horvath, J. Clayton, D.G. Ando, E.E. Sercatz, and L.E. Hood. 1988. Restricted use of T cell receptor V genes in murine autoimmune encephalomyelitis raises possibilities for antibody therapy. *Cell.* 54:577.
26. Lai, M.-Z., S.-Y. Huang, T.J. Briner, J.-G. Guillet, J.A. Smith, and M.L. Gefter. 1988. T cell receptor gene usage in the response to λ-repressor cI protein. An apparent bias in the usage of a Vα gene element. *J. Exp. Med.* 168:1081.
27. Lai, M.-Z., Y.-J. Jang, L.-K. Chen, and M.L. Gefter. 1990. Restricted VDJ junctional regions in the T cell response to λ-repressor. Identification of residues critical for antigen recognition. *J. Immunol.* 144:4851.
28. Taylor, A.H., A.M. Haberman, W. Gerhard, and A.J. Caton. 1990. Structure-function relationships among highly diverse T cells that recognize a determinant from influenzavirus hemagglutinin. *J. Exp. Med.* 172:1643.
29. Yanagi, Y., R. Maekawa, 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.
30. Aebischer, 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.
31. Hochgeschwender, U., H.U. Weltzien, K. Eichman, R.B. Wallace, and J.T. Epplen. 1986. Preferential expression of a defined T-cell receptor β-chain gene in hapten specific cytotoxic T cell clones. *Nature (Lond.).* 322:376.
32. Hochgeschwender, U., H.-G. Simon, H.U. Weltzien, F. Bartels, A. Becker, and J.T. Epplen. 1987. Dominance of one T cell receptor in the H-2K^b TNP response. *Nature (Lond.).* 326: 307.
33. 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.
34. Tan, K.-N., B.M. Datlof, J.A. Gilmore, A.C. Kronman, 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.
35. Romero, P., J.L. Maryanski, G. Corradin, R.S. Nussenzweig, V. Nussenzweig, and F. Zavala. 1989. Cloned cytotoxic T cells recognize an epitope in the circumsporozoite protein and protect against malaria. *Nature (Lond.).* 341:323.
36. Romero, P., J.L. Maryanski, A.S. Cordey, G. Corradin, R. Nussenzweig, and F. Zavala. 1990. Isolation and characterization of protective cytolytic T cells in a rodent malaria model system. *Immunol. Lett.* 25:27.

37. Romero, P., G. Corradin, I.F. Luescher, and J.L. Maryanski. 1991. H-2K^d restricted antigenic peptides share a simple binding motif. *J. Exp. Med.* 174:603.
38. 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.
39. Staerz, U.D., H.-G. Rammensee, J.D. Benedetto, and M.J. Bevan. 1985. Characterization of a murine monoclonal antibody specific for an allotypic determinant on T cell antigen receptor. *J. Immunol.* 134:3994.
40. Haskins, K., C. Hannum, J. White, N. Roehm, R. Kubo, J. Kappler, and P. Marrack. 1984. The antigen-specific major histocompatibility complex-restricted receptor on T cells. An antibody to a receptor allotype. *J. Exp. Med.* 160:452.
41. Payne, J., B.T. Huber, N.A. Cannon, R. Schneider, M.W. Schilham, H. Acha-Orbea, H.R. MacDonald, and H. Hengartner. 1988. Two monoclonal rat antibodies with specificity for the β chain variable region Vβ6 of the murine T-cell receptor. *Proc. Natl. Acad. Sci. USA.* 85:7695.
42. Liao, N.-S., J. Maltzman, and D.H. Raulet. 1989. Positive selection determines T cell receptor Vβ14 gene usage by CD8$^+$ T cells. *J. Exp. Med.* 170:135.
43. Okada, C.Y., B. Holzman, C. Guidos, E. Palmer, and I.L. Weissman. 1990. Characterization of a rat monoclonal antibody specific for a determinant encoded by the Vβ7 gene segment. *J. Immunol.* 144:3473.
44. Louie, M.C., C.A. Nelson, and D.Y. Loh. 1989. Identification and characterization of new murine T cell receptor β chain variable regions (Vβ) genes. *J. Exp. Med.* 170:1987.
45. 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.
46. 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.
47. 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.
48. Alt, F., V. Enea, A.L.M. Bothwell, and D. Baltimore. 1980. Activity of multiple light chain genes in murine myeloma cells producing a single, functional light chain. *Cell.* 21:1.
49. Alt, F., N. Rosenberg, S. Lewis, E. Thomas, and D. Baltimore. 1981. Organization and reorganization of Immunoglobulin genes in A-MuLV-transformed cells: rearrangement of heavy but not light chain genes. *Cell.* 27:381.
50. Blackwell, T.K., and F.W. Alt. 1989. Mechanism and developmental program of Immunoglobulin gene rearrangements in mammals. *Annu. Rev. Genet.* 23:605.
51. Chou, H.S., S.J. Anderson, M.C. Louie, S.A. Godambe, M.R. Pozzi, M.A. Behlke, K. Huppi, and D.Y. Loh. 1987. Tandem linkage and unusual RNA splicing of the T cell receptor β chain variable region genes. *Proc. Natl. Acad. Sci. USA.* 84:1992.
52. Wade, T., J. Bill, P.C. Marrack, E. Palmer, and J.W. Kappler. 1988. Molecular basis for the nonexpression of Vβ17 in some strains of mice. *J. Immunol.* 141:2165.
53. Matis, L.A., A. Ezquerra, and J.E. Coligan. 1988. Expression of two distinct T cell receptor $\alpha\beta$ heterodimers by an antigen-specific T cell clone. *J. Exp. Med.* 168:2379.
54. Schittek, B., E. Unkelbach, and K. Rajewsky. 1989. Violation of allelic exclusion of the T cell receptor β genes in a helper T cell clone. *Int. Immunol.* 1:273.
55. 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.
56. 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.
57. Hayday, A.C., D.J. Diamond, G. Tanigawa, J.S. Heilig, V. Folson, H. Saito, and S. Tonegawa. 1985. Unusual organization and diversity of T cell receptor α chain genes. *Nature (Lond.).* 316:828.
58. Ohashi, P.S., V.A. Wallace, H. Broughton, C.T. Ohashi, D.A. Ferrick, V. Jost, T.M. Mak, H. Hengartner, and H. Pircher. 1990. Specific deletion of the J-Cδ locus in murine $\alpha\beta$ T cell clones and studies using transgenic mice. *Eur. J. Immunol.* 20:517.
59. Malissen, M., J. Trucy, F. Letourneur, N. Rebai, 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.
60. Furutani, M., Y. Yanagi, I. Fujisawa, T. Nakayama, H. Kishimoto, K. Kuida, Y. Asano, and T. Tada. 1989. Post-transcriptional allelic exclusion of two functionally rearranged T cell receptor α genes. *Int. Immunol.* 1:281.
61. Kuida, K., M. Furutani-Seiki, T. Saito, H. Kishimoto, K. Sano, and T. Tada. 1991. Post translational attainment of allelic exclusion of the T cell receptor α chain in a T cell clone. *Int. Immunol.* 3:75.
62. 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.
63. Davis, M.M., and P.J. Bjorkman. 1988. T cell antigen receptor genes and T cell recognition. *Nature (Lond.).* 334:395.
64. Claverie, J.-M., A. Prochnicka-Chalufour, and L. Bougueleret. 1989. Immunological implications of a Fab-like structure for the T cell receptor. *Immunol. Today.* 10:10.
65. Engel, I., and S.M. Hedrick. 1988. Site-directed mutations in the VDJ junctional region of a T cell receptor β chain cause changes in antigenic peptide recognition. *Cell.* 54:473.
66. Guillet, J.-G., M.-Z. Lai, T.J. Briner, S. Buus, A. Sette, H.M. Grey, J.A. Smith, and M.L. Gefter. 1987. Immunological self, nonself discrimination. *Science (Wash. DC).* 235:865.
67. Barth, R.K., B.S. Kim, N.C. Lan, T. Hunkapiller, N. Sobieck, A. Winoto, H. Gershenfeld, C. Okada, D. Hansburg, I.L. Weissman, and L.E. Hood. 1985. The murine T cell receptor uses a limited repertoire of expressed Vβ gene segments. *Nature (Lond.).* 316:517.
68. Behlke, M.A., H.S. Chou, K. Huppi, and D.Y. Loh. 1986. Murine T cell receptor mutants with deletions of β chain variable region genes. *Proc. Natl. Acad. Sci. USA.* 83:767.
69. Singer, P.A., R.J. McEvilly, D.J. Noonan, F.J. Dixon, and A.N. Theofilopoulos. 1986. Clonal diversity and T cell receptor β chain variable gene expression in enlarged lymph nodes of MRL-lpr/lpr lupus mice. *Proc. Natl. Acad. Sci. USA.* 83:7018.
70. 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).* 229:566.

71. 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.
72. 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.
73. 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.
74. Kavaler, J., M.M. Davis, and Y.-H. Chien. 1984. Localization of a T cell receptor diversity region element. *Nature (Lond.).* 310:421.
75. Yague, J., M. Blackman, W. Born, P. Marrack, J. Kappler, and E. Palmer. 1988. The structure of Vα and Jα segments in the mouse. *Nucleic Acids Res.* 16:11355.
76. 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.
77. 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.
78. 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.
79. Chien, Y.-H., M. Iwashima, K.B. Kaplan, J.F. Elliott, and M.M. Davis. 1987. A new T-cell receptor gene located within the alpha locus and expressed early in T-cell differentiation. *Nature (Lond.).* 327:677.
80. Pircher, H., E.E. Michalopoulos, A. Iwamoto, P.S. Ohashi, J. Baenziger, H. Hengartner, R.M. Zinkernagel, and T.W. Mak. 1987. Molecular analysis of the antigen receptor of virus-specific cytolytic T cells and identification of a new Vα family. *Eur. J. Immunol.* 17:1843.
81. Korman, A., S. Marusic-Galesic, D. Spencer, A. Kruisbeck, and D.H. Raulet. 1988. Predominant variable region gene usage by γ-δ T cell receptor-bearing cells in the adult thymus. *J. Exp. Med.* 168:1021.
82. Okazaki, K., and H. Sakano. 1988. Thymocyte circular DNA excised from T cell receptor α-δ gene complex. *EMBO (Eur. Mol. Biol. Organ.) J.* 7:1669.
83. Chien, Y.-H., D.M. Becker, T. Lindsten, M. Okamura, D.I. Cohen, and M.M. Davis. 1984. A third type of murine T cell receptor gene. *Nature (Lond.).* 312:31.
84. Dembic, Z., W. Haas, S. Weiss, J. McCubrey, H. Kiefer, H. von Boehmer, and M. Steinmetz. 1986. Transfer of specificity by murine α and β T cell receptor genes. *Nature (Lond.).* 320:232.
85. Marolleau, J.-P., J.D. Fondell, M. Malissen, J. Trucy, E. Barbier, K.B. Marcu, P.A. Cazenave, and D. Primi. 1988. The joining of germ-line Vα to Jα genes replaces the preexisting Vα-Jα complexes in a T cell receptor $\alpha\beta$ positive T cell line. *Cell.* 55:291.
86. Fondell, J.D., J.-P. Marolleau, D. Primi, and K. Marcu. 1990. On the mechanism of non-allelically excluded Vα-Jα T cell receptor secondary rearrangements in a murine T cell lymphoma. *J. Immunol.* 144:1094.