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Restricted V-Segment Usage in T-Cell Receptors from Cytotoxic T Lymphocytes Specific for a Major Epitope of Lymphocytic Choriomeningitis Virus†

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Cytotoxic T lymphocytes (CTL) play an important role in recovery from a number of viral infections. They are also implicated in virus-induced immunopathology, as best demonstrated in lymphocytic choriomeningitis virus (LCMV) infection of adult immunocompetent mice. In the present study, the structure of the T-cell receptor (TCR) in LCMV-specific CTL in C57BL/6 (B6) mice was investigated. Spleen T cells obtained from LCMV-infected mice were cultured in vitro with virus-infected stimulator cells and then stained with anti-TCR V_β antibodies. A skewing of V_β usage was noticeable in T cells enriched for their reactivity to LCMV, suggesting that particular V segments are important for the recognition of LCMV T-cell epitopes in B6 mice. To gain more detailed information on the structure of the TCR specific for LCMV epitopes, we studied CTL clones. It has been shown that approximately 90% of LCMV-reactive CTL clones generated in *H-2[b]* mice are specific for a short peptide fragment of the LCMV glycoprotein, residues 278 to 286, recognized in the context of the class I major histocompatibility complex molecule, D^b. Four CTL clones possessing this specificity were randomly selected from a collection of clones, and their TCR genes were isolated by cDNA cloning or by the anchored polymerase chain reaction. All four clones were found to use V_α gene segments belonging to the $V_\alpha4$ subfamily. By RNA blot analysis, two more clones with the same specificity were also shown to express the $V_\alpha4$ mRNA. In contrast, three different V_β gene segments were used among the four clones examined. $J_\beta2.1$ was used by three of the clones. Although amino acid sequences in the V(D)J junctional regions were dissimilar, aspartic acid was found in the $V_\alpha J_\alpha$ and/or $V_\beta D_\beta J_\beta$ junctions of all four of these clones, suggesting that this residue is involved in binding the LCMV fragment. Restricted usage of V_α and possibly J_β segments in the CTL response to a major T-cell epitope of LCMV raises the possibility that immunopathology in LCMV infection can be treated with antibodies directed against such TCR segments. Thus, similar analysis of the TCR in other virus infections is warranted and may lead to therapeutic strategies for immunopathology due to virus infections.

T cells recognize foreign antigen in the context of self major histocompatibility complex (MHC) molecules (58). In general, cytotoxic T lymphocytes (CTL) recognize antigens in association with class I MHC molecules, whereas helper T cells see antigens associated with class II MHC molecules. The processed or degraded antigen peptides appear to be bound to MHC molecules in a groove between the α-helices of the polymorphic domains of MHC molecules (8, 9, 50). The complex of a peptide fragment and an MHC molecule thus formed is recognized by a membrane-bound T-cell receptor (TCR) composed of α and β chains (16). The TCR α and β chains are encoded by discontinuous variable (V), diversity (D), joining (J), and constant (C) gene segments (13, 22, 42, 56). The V, D, and J gene segments are rearranged during T-cell ontogeny to form a functional, continuous V-region gene, which is then posttranscriptionally spliced to C-region sequences. As with immunoglobulin genes, this mechanism of rearrangement in TCR genes can generate the high degree of receptor diversity required to recognize and respond to a virtually limitless universe of antigens (16).

Several groups have examined the relationship of TCR structure to function by characterizing the TCR sequences of T-cell clones and hybridomas with related antigen specificities. In some cases, such as for pigeon cytochrome *c*-specific clones, the majority of the T cells with the same antigen and MHC specificity were found to use the same α- and β-chain gene segments (19, 24, 54). In the response to arsonate, most T cells use a particular α-chain V-gene segment ($V_\alpha3$), independent of the MHC restriction elements used (49). In other cases, however, the TCR sequences are more heterogeneous (25, 46, 47). On the basis of analysis of TCR sequences, intervention in the immune response has been attempted by using TCR-specific antibodies. For example, the vast majority of the T-cell clones causing autoimmune encephalomyelitis in PL and B10.PL mice were found to have the same V_α and V_β gene segments, and an antibody to the V_β segment expressed by these T cells was successfully used to prevent the development of autoimmune encephalitis (1, 51). A similar treatment, however, was not successful in a different strain of mouse (SJL), in which the response to myelin basic protein is more complex (43).

In this report, the structure of the TCR in virus-specific CTL was studied. We chose the T-cell response to lympho-

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cytic choriomeningitis virus (LCMV) as a model system. LCMV is a member of the arenaviruses (bisegmented ambisense RNA viruses) (7). The short (S) RNA encodes two major structural proteins, glycoprotein (GP) and nucleoprotein (NP), which are primarily responsible for induction of protective humoral (33) and cell-mediated (35) immunity. When the virus is injected intravenously or intraperitoneally, adult mice eliminate virus and recover. In contrast, when the virus is injected intracerebrally, the mice develop severe meningitis and ventriculitis and die 7 to 10 days later. Because death is attributable to the mouse's own vigorous cell-mediated immune response to virally infected leptomeningeal and ventricular cells, it can be prevented by immunosuppression (11, 29, 38). Both virus clearance (immunity) and virus-induced immunopathology are mediated by $CD8^+$ LCMV-specific class I MHC-restricted CTL (11, 57). T-cell epitopes and restricting MHC molecules have been characterized in detail in the CTL response to LCMV (32, 45, 52, 53), making this model system suitable for the study of TCR. Analysis of the TCR in virus-specific T cells should provide important information not only on the structure-function relationship of T-cell recognition but also on the potential for manipulation and abrogation of the harmful immune response to viral infections.

MATERIALS AND METHODS

Virus stock. The Armstrong CA1371 strain clone 53b (ARM) of LCMV was used for this study (17).

Mice. Male C57BL/6 (B6) mice, 6 to 8 weeks of age, were obtained from the breeding colony at the Research Institute of Scripps Clinic. LCMV infection was induced by intraperitoneal injection of 2×10^5 PFU.

CTL clones. Isolation and maintenance of LCMV-specific CTL clones used in this study were described previously (12, 32, 52).

Antibodies and cell staining. The following murine TCR V_β-specific antibodies were used in these experiments: MR9-4 ($V_\beta5$), RR4-7 ($V_\beta6$) (27), KJ16 ($V_\beta8.1$ and $V_\beta8.2$) (37), F23.1 ($V_\beta8$) (48), MR10-2 ($V_\beta9$), and RR3-15 ($V_\beta11$) (6). KJ16 and F23.1 were obtained from P. Marrack, National Jewish Center for Immunology and Respiratory Medicine, Denver, Colo., and M. Bevan, Research Institute of Scripps Clinic, La Jolla, Calif., respectively. MR9-4 and MR10-2 were generated by one of us (O. Kanagawa, unpublished data). Other antibodies used were 30-H12 (Thy1.2), YTS169.4 (CD8), and YTS191.1 (CD4). MR9-4, F23.1, and MR10-2 are mouse immunoglobulin G, and the others are rat immunoglobulin G.

Immunofluorescence staining was carried out by following a standard protocol. Briefly, 3×10^5 to 5×10^5 cells were washed with medium containing 2% serum and incubated at 4°C for 30 min with an individual antibody (100 μl of culture supernatant from an antibody-producing hybridoma). After being washed three times, the cells were incubated with fluorescein isothiocyanate-labeled anti-immunoglobulin (anti-rat immunoglobulin or anti-mouse immunoglobulin depending on the first antibody used) at 4°C for 30 min, washed three times again, and resuspended in phosphate-buffered saline. Fluorescein isothiocyanate-labeled anti-immunoglobulin antibodies were purchased from Boehringer Mannheim, Indianapolis, Ind. Fluorescence-activated cell sorter analysis was performed on a FACS-IV machine.

In vitro enrichment of LCMV-specific CTL. Spleen cells were taken from B6 mice infected with 2×10^5 PFU of LCMV 7 days or 3 months previously. These spleen cells were stimulated with syngeneic peritoneal exudate cells (PEC) infected with LCMV (used 48 h postinfection at a multiplicity of infection of 2) and subjected to 2,000 rads of irradiation. Then 8×10^6 spleen cells and 1.5×10^5 infected and irradiated PEC were placed in each 16-mm well of 24-well Costar plates in 2 ml of RPMI 1640 medium supplemented with 10% fetal calf serum, 1 mM glutamine, 5×10^{-5} M β-mercaptoethanol, penicillin, and streptomycin (12). After in vitro culture, the spleen cells were depleted of B cells and stained with antibodies. The B-cell depletion was done by a panning method with a plate coated with anti-mouse immunoglobulin (55). This method depleted more than 95% of B cells as revealed by fluorescence-activated cell sorter analysis. Some of the spleen cells were further stimulated weekly for another 3 weeks and in the presence of T-cell growth factor for the last 2 weeks. T-cell growth factor was prepared by treating Lewis rat lymphocytes with concanavalin A. $CD8^+$ T cells were enriched from B-cell-depleted spleen cells taken from uninfected mice by using the panning procedure described by Nikolic-Zugic and Bevan (31).

cDNA library construction, isolation of TCR genes, and nucleotide sequencing. Cytoplasmic RNA was extracted from the T-cell clones, and double-stranded cDNA was synthesized from 10 μg of total RNA by the method of Gubler and Hoffman (21). The ends of cDNA were blunted with mung bean nuclease and the large fragment of DNA polymerase I (20). cDNA larger than 500 bp possessing *Eco*RI linkers was ligated to *Eco*RI-digested λ gt10. After in vitro packaging, recombinants were plated onto a lawn of bacteria and screened with ^{32}P-labeled DNA probes of TCR α- and β-chain C regions (25) (generously provided by T. W. Mak, Ontario Cancer Institute, Toronto, Canada). cDNA inserts of positive clones were isolated from λ gt10-cDNA recombinants and subcloned into a plasmid vector, pUC18. Nucleotide sequences of the cDNA inserts were determined by the dideoxy-chain termination method (44).

Anchored polymerase chain reaction. The polymerase chain reaction (PCR) procedure described by Loh et al. (30) was followed with some modifications. The first-strand cDNA was synthesized from 10 μg of total RNA by using reverse transcriptase and oligo(dT) primer. [^3H]dGTP was included in the reaction to trace the synthesis of the cDNA. To eliminate the oligo(dT) primer, which is inhibitory in the next step of the reaction (tailing), we electrophoresed cDNA/mRNA hybrids in low-melting-point agarose. cDNA/mRNA hybrids greater than 500 bp were recovered from the gel, purified, and then precipitated in ethanol. A poly(dG) tail was added to the 3′ end of the cDNA with terminal deoxynucleotidyltransferase (TdT) and dGTP (28). [^3H]dGTP was also included in the reaction to enable the length of the dG tail, which should be 10 to 20 G residues, to be ascertained. The product was then amplified by the PCR (40) by using the TCR C-region primer (C_α or C_β) and another oligonucleotide consisting of a poly(dC) tail attached to a sequence containing convenient restriction sites, termed the anchor (AN). The AN primer [without the attached poly(dC) tail] was also included to overcome the potential problem of generating progressively longer stretches of poly(dG)-poly(dC) with each cycle of the PCR (30). The AN poly(C) primer (5′-CCTCGAGATCCTGCAGAATTCCCCCCCCCC CCCC-3′) and the AN primer (5′-CCTCGAGATCCTGCA GAATTCC-3′) contain *Xho*I, *Xho*II, *Pst*I, and *Eco*RI restriction sites. The C_α primer (5′-GCTCGAGGATCTTTT AACTGGTACA-3′) and the C_β primer (5′-ACTCGAGTCA CATTTCTCAGATCCT-3′) possess the *Xho*I site, which

TABLE 1. TCR V_β usage in C57BL/6 mouse spleen T cells enriched for reactivity to LCMV[a]

Antibody	% V_β usage in spleen cells from uninfected mice		% V_β usage in spleen cells taken from mice infected 7 days previously; in vitro stimulation at:		% V_β usage in spleen cells taken from mice infected 3 mo previously; in vitro stimulation at:	
	Unfractionated	CD8$^+$ enriched	1 wk	4 wk	1 wk	4 wk
Thy-1	91.4	96.2	94.1	96.3	98.1	94.7
CD8	25.2	88.6	36.4	97.7	88.2	98.7
CD4	58.2	5.7	34.2	0	11.1	0.4
Immunoglobulin	1.3	1.8	1.3	1.6	2.2	1.4
$V_\beta5$	6.5	11.9	6.9	0	4.1	0
$V_\beta6$	5.3	7.3	7.3	8.8	6.2	1.9
$V_\beta8$ (F23.1)	16.9	ND[b]	19.1	ND	21.0	ND
$V_\beta8$ (KJ16)	14.5	14.3	16.2	21.8	18.2	9.2
$V_\beta9$	1.9	3.5	1.6	1.5	4.1	13.1
$V_\beta11$	4.3	9.7	6.0	0.7	6.3	2.9

[a] When anti-mouse immunoglobulin was used as the second antibody, the percentage shown in the table was obtained by subtracting the frequency of immunoglobulin-positive cells (B cells) from that of the cells positively stained with a given first antibody.
[b] ND, Not done.

was created by modifying the respective authentic sequences. Amplification was carried out for 30 cycles. The product was electrophoresed in 1% low-melting-point agarose, and the amplified DNA fragment (ca. 550 bp) was recovered. The purified DNA was digested with the restriction enzymes *Eco*RI and *Xho*I and then cloned into the plasmid vector, pUC18 digested with *Eco*RI and *Sal*I. Nucleotide sequences of inserts were determined by the dideoxy-chain termination method (44).

RNA blot analysis. Total RNA isolated from cultured cells (10 μg each) was treated with glyoxal and electrophoresed through a 1% agarose gel in 10 mM sodium phosphate buffer (pH 7) (20). After the RNA had been transferred to nitrocellulose filters, hybridization was carried out in 50% formamide–5× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate)–1× Denhardt solution–10% dextran sulfate–250 μg of denatured salmon sperm DNA per ml–1 × 10^6 cpm of ^{32}P-labeled $V_\alpha4$ probe per ml at 42°C for 20 h. The $V_\alpha4$ probe used was a 270-bp *Sau*3A fragment derived from the TCR α-chain gene of HL228. The filters were washed twice in 1× SSC at 50°C for 15 min each and autoradiographed with X-ray film and intensifying screens.

RESULTS

V_β usage in the bulk T-cell response to LCMV. To determine whether there is any correlation between the TCR structure and the specificity for LCMV, and whether the T-cell response to LCMV is homogeneous or not, we generated in vitro cultures enriched for LCMV-specific T cells. We enriched virus-specific CTL from spleen cells of acutely infected mice (infected 7 days previously) as well as from spleen cells of immune mice (infected 3 months previously). Spleen cells harvested from LCMV-infected B6 mice were cultured in vitro together with LCMV-infected irradiated macrophages as stimulator cells. After in vitro culture, lymphocytes were depleted of B cells and stained with anti-TCR V_β antibodies. In these experiments, we used only anti-V_β antibodies specific for certain V_β subfamilies, as antibodies are not available against all V_β subfamilies and no V_α subfamily-specific antibodies have yet been described. When the in vitro culture was initiated for the spleen cells harvested from B6 mice infected 7 days previously, no skewing of V_β usage was evident following 1 week of culture. However, the proportion of CD8$^+$ cells was moderately increased (Table 1). The picture became very different following weekly stimulation with LCMV-infected macrophages for another 3 weeks. At this time almost 100% of cultured cells were CD8$^+$; the percentages of $V_\beta5^+$ and $V_\beta11^+$ cells were greatly reduced (Table 1). The experiment was repeated with spleen cells harvested from B6 mice infected 3 months previously. In this case, enrichment of CD8$^+$ cells was already prominent after 1 week of culture. The percentages of $V_\beta5^+$ and $V_\beta11^+$ cells were again greatly reduced following 4 weeks of stimulation. In addition, the proportions of $V_\beta6^+$ and $V_\beta8^+$ cells were similarly reduced, while in contrast, the percentage of $V_\beta9^+$ cells was increased fourfold compared with that in CD8$^+$ spleen cells from uninfected mice. (Since there are some differences in V_β usage between CD4$^+$ and CD8$^+$ T cells, V_β usages in both unfractionated and CD8$^+$-enriched spleen T cells from uninfected B6 mice are presented in Table 1 as controls.) The skewed V_β usage observed in these cultures suggests the presence of predominant T-cell clones specific for LCMV in B6 mice, although the incomplete availability of TCR V segment-specific antibodies precludes the identification of those clones.

Structure of TCR in LCMV-specific CTL clones. To further characterize the structure of TCR involved in the recognition of LCMV, we randomly chose four LCMV-specific CTL clones, HL31, RG9, HL228, and HL39 (52), for the determination of TCR sequences. These clones are derived from three independent cloning experiments (HL31 and HL39 are derived from the same set of experiments) and were shown to have different patterns of gene rearrangement of the TCR β chain (26). Reactivities of these clones have been reported previously (32, 52) and are summarized in Table 2. All of the clones recognize the LCMV GP amino acid residues 278 to 286 in association with the class I MHC molecule, D^b. It has been shown that approximately 90% of CTL clones generated in the *H-2^b* haplotype are specific for this GP fragment and are restricted by D^b (32, 52). Although these clones are all specific for the same nine amino acid residues of GP, the reactivities to peptides with single-amino-acid substitutions at position 278 are different among these four clones (32) (Table 2). HL228 failed to respond to target cells coated with the peptide having the valine 278-to-serine or threonine substitution, whereas the other clones retained the reactivity. The valine 278-to-phenylalanine substitution caused HL39 to lose its reactivity. All of the clones either lost

TABLE 2. Reactivities of LCMV-specific CTL clones

Target cells inoculated with:	Reactivity of:			
	HL31	RG9	HL228	HL39
H-2^{b} target				
LCMV	+	+	+	+
VV-GP[a]	+	+	+	+
VV-NP[a]	−	−	−	−
Peptide GP 1 to 16	−	−	−	−
Peptide GP 278 to 286	+	+	+	+
Peptide GP 272 to 293	+	+	+	+
Peptide GP 272 to 293 with single substitution at position 278[b]				
V → L, I, or A	+	+	+	+
V → S or T	+	+	−	+
V → F	+	+	−	−
V → Y, D, or E	±	±	−	−
H-2^{d} target				
LCMV	−	−	−	−

[a] VV-GP and VV-NP are vaccinia virus recombinants expressing the LCMV (GP) residues 1 to 363 and the LCMV NP residues 1 to 558, respectively.

[b] Leucine (L), isoleucine (I), alanine (A), serine (S), threonine (T), phenylalanine (F), tyrosine (Y), aspartic acid (D), or glutamic acid (E) is substituted for valine (V) at position 278 of the LCMV GP 272 to 293 peptide.

reactivity or had reduced reactivity to the peptide when valine 278 was substituted by tyrosine, aspartic acid, or glutamic acid.

A cDNA library was constructed from cytoplasmic RNA of HL228, and genes encoding TCR α and β chains were molecularly cloned. The TCR genes of the remaining CTL clones were obtained by using the anchored PCR (30) in which the first-strand cDNA was tailed by using TdT and oligo(dG) and then V-region genes were amplified by using oligo(dC) and TCR C-region primers. Nucleotide sequences of cloned TCR genes were determined and compared with the reported TCR sequences. At least three independent clones of each TCR gene were sequenced to verify nucleotide sequences and in-frame gene rearrangements. We found only one type of mRNA for each gene from all the CTL clones analyzed. The results are summarized in Table 3. RG9 and HL228 share the same α-chain sequence, V_{α}4.HL228, J_{α}.HL228 (Fig. 1). V_{α}4.HL228 has not been reported previously and has a 99.4% similarity at the nucleotide level to V_{α}4.DA33 (47). There are two nucleotide differences between these sequences, both of which lead to an amino acid substitution. Since no sequences of the corresponding genomic DNA are available, we cannot rule out the possibility that the differences are due to somatic mutations or allelic differences (even though T-cell clones from which the TCR genes were isolated are both derived from B6 mice). J_{α}.HL228 also has not been previously reported. HL31 and HL39 have the same V_{α} (V_{α}4.TA65) (3), but use different J_{α} segments (3, 54). Thus, all of the CTL clones examined here use gene segments belonging to the V_{α}4 subfamily. In contrast, three different V_{β} gene segments, V_{β}7, V_{β}9, and V_{β}10 (5, 41), are used among the four clones. V_{β}10 is used by RG9 and HL39. Three clones use J_{β}2.1, and the other (HL39) uses J_{β}2.6 (14). Four of the five highly variable amino acid residues for 12 J_{β} segments are shared by J_{β}2.1 and J_{β}2.6 (Fig. 2). J_{β}2.6 is missing alanine and has a conservative phenylalanine-to-tyrosine substitution in comparison with J_{β}2.1. Otherwise, J_{β}2.1 and J_{β}2.6 are

TABLE 3. TCR sequences of LCMV-specific CTL clones

CTL clone	TCR α-chain sequence	TCR β-chain sequence
HL31	V_{α}4.TA65 J_{α}.TA31	V_{β}7 J_{β}2.1
RG9	V_{α}4.HL228 J_{α}.HL228	V_{β}10 J_{β}2.1
HL228	V_{α}4.HL228 J_{α}.HL228	V_{β}9 J_{β}2.1
HL39	V_{α}4.TA65 J_{α}.14.4	V_{β}10 J_{β}2.6

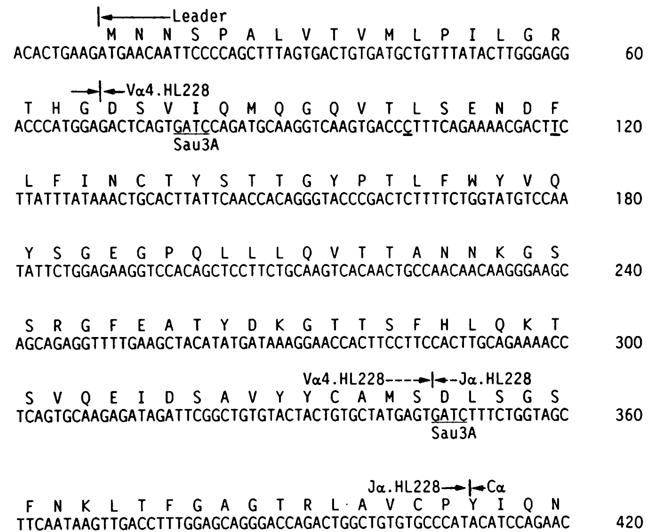

FIG. 1. Nucleotide and predicted amino acid sequences of the TCR α chain from LCMV-specific CTL clones, HL228 and RG9. The boundary between the V_{α} and J_{α} segments cannot be assigned unequivocally as nucleotide sequences of the genomic DNA encoding V_{α}4.HL228 and J_{α}.HL228 are not available. Nucleotides that differ from the corresponding nucleotide in V_{α}4.DA33 are underlined. The *Sau*3A restriction sites used to prepare the V_{α}4-specific probe are indicated.

		*	*	*	*				*							
J_{β}2.1	N	Y	A	E	Q	F	F	G	P	G	T	R	L	T	V	L
2.2	.	T	G	Q	L	Y	.	.	E	.	S	K	.	.	.	.
2.3	S	A	E	T	L	Y	.	.	S	.	.	.	.	.	.	.
2.4	S	Q	N	T	L	Y	.	.	A	.	.	.	.	S	.	.
2.5	.	Q	D	T	.	Y	.	.	.	.	.	.	.	L	.	.
2.6	S	.		.	.	Y	.	.	.	.	.	.	.	.	.	.
1.1		N	T	.	V	.	.	.	K	.	.	.	.	.	.	V
1.2		N	S	D	Y	T	.	.	S	.	.	.	.	L	.	I
1.3	S	G	N	T	L	Y	.	.	E	.	S	.	.	I	.	V
1.4	S	N	E	R	L	.	.	.	H	.	.	K	.	S	.	.
1.5		Q	P	A	P	L	.	.	E	.	.	.	.	S	.	.
1.6	Y	N	S	P	L	Y	.	A	A	.	.	.	.	.	.	T

FIG. 2. Comparison of amino acid sequences among the 12 J_{β} regions. Symbols: •, residues identical to the corresponding residues in J_{β}2.1; *, highly variable residues. The first amino acid residues shown are often replaced by other residues depending on how the J_{β} gene segments are joined to the D_{β} gene segments.

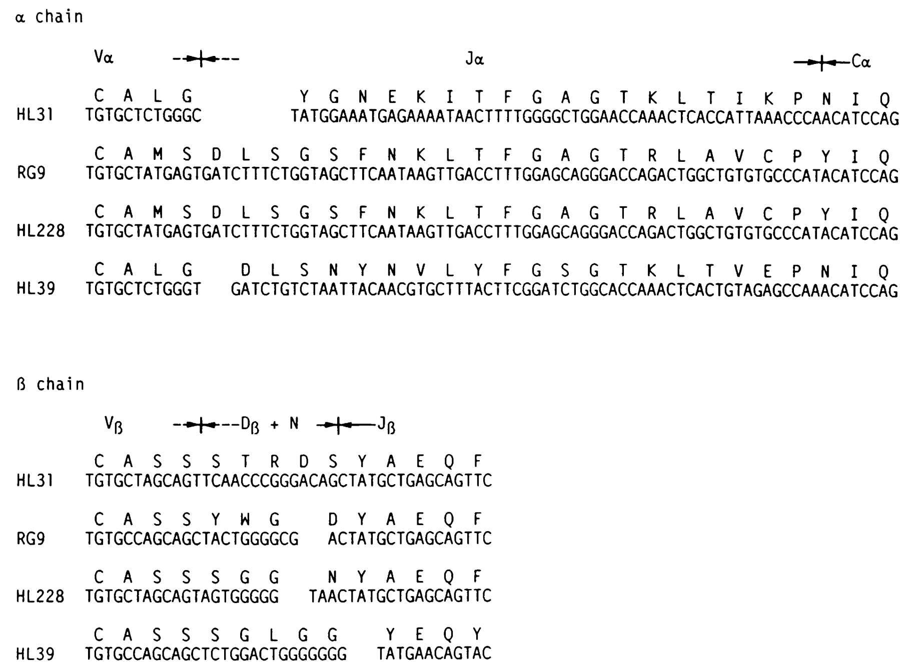

FIG. 3. Nucleotide and predicted amino acid sequences around the V(D)J junctional region of the TCR α and β chains from four LCMV-specific CTL clones. Complete sequences are shown for the J_α region, whereas only partial J_β sequences are shown, as the rest of the amino acid sequences are identical between $J_\beta2.1$ and $J_\beta2.6$. N sequences (N) added without templates during gene rearrangement are also indicated. Note the consistent presence of aspartic acid (D) in the $V_\alpha J_\alpha$ and/or $V_\beta D_\beta J_\beta$ junctions of each CTL clone.

identical. Thus, the sequence in the J_β region may also be restricted in the CTL clones specific for this epitope of LCMV. Nucleotide and predicted amino acid sequences in the V(D)J junctional regions of the four clones are shown in Fig. 3. On the basis of sequence similarities with immunoglobulin, it has been proposed that the tertiary structure of the TCR αβ heterodimer is very similar to that of the immunoglobulin Fab fragment, that complementarity-determining region 1 (CDR1)- and CDR2-equivalent regions on V_α and V_β contact the side chains of the MHC α-helices, and that the centrally located CDR3-equivalent regions contact the bound peptide (15, 16). The CDR3-equivalent regions correspond to the V(D)J junctional regions of the TCR: the V_α-J_α junction and the beginning of the J_α region in the α chain and the V_β-D_β and D_β-J_β junctions, the D_β region, and the beginning of the J_β region in the β chain (16). Although no particular pattern(s) of amino acid residues was apparent in the $V_\alpha J_\alpha$ and $V_\beta D_\beta J_\beta$ junctional regions, the consistent presence of aspartic acid in the junctions was notable (at position 93 of the α chain for RG9, HL228, and HL39 and at position 98 of the β chain for HL31 and RG9). This amino acid residue might be important in binding the LCMV peptide fragment. The length of the $V_\beta D_\beta J_\beta$ junctional region is almost the same among the clones (HL31 has the junction one residue longer than the others), whereas the length of the $V_\alpha J_\alpha$ junctional region is more variable (up to a three-residue difference).

To confirm whether members of the $V_\alpha4$ subfamily are frequently used among CTL clones specific for the LCMV GP 278 to 286 epitope in the *H-2*^{b} haplotype, we analyzed two more CTL clones with this specificity, HL232 and HL36 (26, 52), for their V_α usage by RNA blot analysis. These clones also expressed the $V_\alpha4$ mRNA like the four clones analyzed above, whereas other CTL clones specific for the LCMV NP and L^d, HD8, and HD32 (53), did not express the $V_\alpha4$ gene (Fig. 4).

DISCUSSION

In this paper we report that six independent CTL clones specific for the immunodominant T-cell epitope of LCMV in the *H-2*^{b} haplotype all use gene segments of the $V_\alpha4$ subfamily. Three of four CTL clones examined in detail use $J_\beta2.1$, and the other uses $J_\beta2.6$. Thus, the gene usage for V_α and possibly J_β seems restricted among CTL clones specific for this epitope of LCMV. Pircher et al. reported that an LCMV-specific D^b-restricted CTL clone (P14) used $V_\alpha2$,

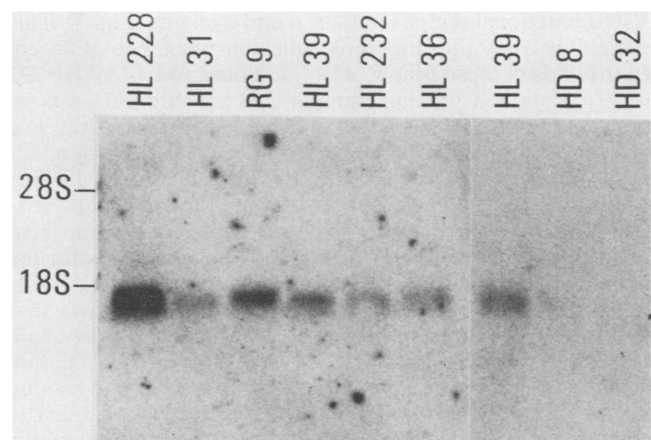

FIG. 4. RNA blot analysis of LCMV-specific CTL clones in an examination of the expression of the $V_\alpha4$ mRNA.

J_α.TA31, V_β8.1, and J_β2.4 (34), although they did not define the epitope recognized by this clone. In addition to GP 278 to 286, we have mapped two other CTL epitopes of LCMV in the *H-2^b* haplotype. These include an epitope at GP 34 to 42, restricted by D^b, and an epitope at NP 397 to 407 (M. B. A. Oldstone and A. Tishon, unpublished results). It is possible that P14 is specific for either of these two epitopes, and not for GP 278 to 286. In contrast to the consistency of V_α4 usage, V_β usage was more variable and was not restricted among the CTL clones. In the bulk population of LCMV-reactive T cells, the percentages of $V_\beta5^+$ and $V_\beta11^+$ cells were greatly reduced (Table 1), allowing T cells with other V_βs to compensate for their underrepresentation. V_β7 and V_β10 used in the three LCMV-specific CTL clones might be used by such T cells. This can be tested directly when antibodies specific for V_β7 and V_β10 become available. $V_\beta9^+$ cell numbers were increased in in vitro stimulated spleen cells taken from mice infected 3 months previously. This is consistent with our finding that one of the CTL clones uses V_β9. Thus, it is possible that the V_β usage is partially restricted to a few particular V_βs. This possibility is currently under study, using a larger number of CTL clones. During the preparation of this paper, we became aware that another group also analyzed the TCR of D^b-restricted CTL clones specific for LCMV GP 275 to 289. Although they have determined nucleotide sequences of the TCR for only one CTL clone, they found by RNA blot analysis that all five CTL clones examined expressed V_α4 and V_β10 genes (2). Our results agree with theirs for the restricted usage of the V_α4 subfamily, but disagree for the restricted usage of V_β10. The reason for this discrepancy in V_β usage is not known.

It has been proposed that CDR1- and CDR2-equivalent regions on V_α and V_β segments are responsible for binding the MHC molecule, whereas CDR3-equivalent regions composed of the $V_\alpha J_\alpha$ and $V_\beta D_\beta J_\beta$ junctional regions account for binding the antigenic peptide fragment (15, 16). Then, the consistent usage of the V_α4 subfamily in the CTL clones examined might suggest that V_α4 has a preferential affinity for the class I MHC molecule, D^b. However, a survey of reported TCR genes from D^b-restricted or D^b-reactive T cells indicates otherwise. Such cells are reported to use V_α1 (25), V_α2 (34), V_α3 (10, 25), V_α5 (4), and V_α8 (39). Therefore, the V_α4 segment itself cannot account for the specificity for D^b. However, it is possible that the D^b molecule complexed with the specific viral peptide, LCMV GP 278 to 286, forms a structure that best binds the V_α4 segment. The four CTL clones examined had dissimilar amino acid sequences in the V(D)J junctional regions of their α and β chains (Fig. 3). This variability may be the molecular basis of the different reactivities of these four $V_\alpha4^+$ CTL clones to LCMV GP 272 to 293 peptides with single-amino-acid substitutions at position 278 (Table 2). Hedrick et al. have reported the presence of a highly selected junctional-region residue, found at position 100 of the β chain, in TCRs from pigeon cytochrome *c*-specific T-cell clones (23). Using site-directed mutagenesis, they have also shown that this conserved amino acid residue plays a key role in determining the specificity for the antigenic peptide (18). Closer examination of V(D)J junctional regions of the CTL clones studied here shows that aspartic acid is present at position 93 of the TCR α chain from RG9, HL228, and HL39. HL31 does not contain aspartic acid in the $V_\alpha J_\alpha$ junction, but does have that residue in the $V_\beta D_\beta J_\beta$ junction. The conserved presence of aspartic acid within V(D)J junctions of these clones suggests that this amino acid residue is important in binding the LCMV antigenic fragment.

Finally, an important finding that may have immunotherapeutic implication is that six D^b-restricted CTL clones reactive to the major T-cell epitope of LCMV use a particular V_α subfamily. Therefore, it might be possible to manipulate the T-cell response to LCMV in B6 or *H-2^b* mice by administering antibodies against the V_α4 segment if $V_\alpha4^+$ cells indeed play a major role in the in vivo CTL response. Interestingly, Rodewald et al. recently reported a similar situation of restricted TCRs specific for murine cytomegalovirus, wherein the CTL precursors specific for the immediate-early protein pp89 of murine cytomegalovirus in BALB/c mice are three- to fivefold more frequent in $V_\beta8^+$ T cells than in $V_\beta8^-$ T cells (36), again presenting the potential for possible intervention of the immune response by using TCR V segment-specific antibodies. This specific immune intervention would be of extreme importance in a variety of viral diseases, in which the main clinical manifestations are due to the virus-specific T-cell response rather than to damage directly caused by the virus and for which no specific therapeutic regimen exists. To test this contention, we are now preparing anti-V_α4 antibodies to examine whether treatment of LCMV-infected mice with those antibodies will prevent them from developing a usually lethal virus-induced immunopathologic disease.

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LITERATURE CITED

1. **Acha-Orbea, H., D. J. Mitchell, L. Timmermann, D. C. Wraith, G. S. Tausch, M. K. Waldor, S. S. Zanvil, H. O. McDevitt, and L. Steinman.** 1988. Limited heterogeneity of T cell receptors from lymphocytes mediating autoimmune encephalomyelitis allows specific immune intervention. Cell **54:**263–273.
2. **Aebischer, T., S. Oehen, and H. Hengartner.** 1990. Preferential usage of V_α4 and V_β10 T cell receptor genes by lymphocytic choriomeningitis virus glycoprotein-specific H-2 D^b-restricted cytotoxic T cells. Eur. J. Immunol. **20:**523–531.
3. **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 (London) **316:**783–787.
4. **Becker, D. M., P. Patten, Y.-H. Chien, T. Yokota, Z. Eshhar, M. Gieldin, N. R. J. Gascoigne, C. Goodnow, R. Wolf, K. Arai, and M. M. Davis** 1985. Variability and repertoire size of T-cell receptor V_α gene segments. Nature (London) **317:**430–434.
5. **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 **229:**566–570.
6. **Bill, J., O. Kanagawa, D. Woodland, and E. Palmer.** 1989. The MHC molecule I-E is necessary but not sufficient for the clonal deletion of V_β11-bearing T cells. J. Exp. Med. **169:**1405–1419.
7. **Bishop, D. H. L.** 1990. Arenaviridae and their replication, p. 1231–1243. *In* B. N. Fields, D. M. Knipe, R. M. Chanock, M. S. Hirsch, J. L. Melnick, T. P. Monath, and B. Rolzman (ed.), Virology, 2nd ed. Raven Press, New York.
8. **Bjorkman, 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 (London) **329:**506–512.
9. **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 I histocompatibility

antigens. Nature (London) 329:512–518.

10. **Bluthmann, H., P. Kisielow, Y. Uematsu, M. Malissen, P. Krimpenfort, A. Berns, H. von Boehmer, and M. Steinmetz.** 1988. T-cell-specific deletion of T-cell receptor transgenes allows functional rearrangement of endogenous α- and β-genes. Nature (London) 334:156–159.
11. **Buchmeier, M. J., R. M. Welsh, F. J. Dutko, and M. B. A. Oldstone.** 1980. The virology and immunobiology of lymphocytic choriomeningitis virus infection. Adv. Immunol. 30:275–331.
12. **Byrne, J. A., R. Ahmed, and M. B. A. Oldstone.** 1984. Biology of cloned cytotoxic T lymphocytes specific for lymphocytic choriomeningitis virus. I. Generation and recognition of virus strains and H-2^b mutants. J. Immunol. 133:433–439.
13. **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 (London) 312:31–35.
14. **Chien, Y.-H., N. R. J. Gascoigne, J. Kavaler, N. E. Lee, and M. M. Davis.** 1984. Somatic recombination in a murine T-cell receptor gene. Nature (London) 309:322–326.
15. **Chothia, C., D. R. Boswell, and A. M. Lesk.** 1988. The outline structure of the T-cell αβ receptor. EMBO J. 7:3745–3755.
16. **Davis, M. M., and P. J. Bjorkman.** 1988. T-cell antigen receptor genes and T-cell recognition. Nature (London) 334:395–402.
17. **Dutko, F. J., and M. B. A. Oldstone.** 1983. Genomic and biological variation among commonly used lymphocytic choriomeningitis virus strains. J. Gen. Virol. 64:1689–1698.
18. **Engel, I., and S. M. Hedrick.** 1988. Site-directed mutations in the V D J junctional region of a T cell receptor β chain cause changes in antigenic peptide recognition. Cell 54:473–484.
19. **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 (London) 321:219–226.
20. **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. 1:281–288.
21. **Gubler, U., and B. J. Hoffman.** 1983. A simple and very efficient method for generating cDNA libraries. Gene 25:263–269.
22. **Hedrick, S. M., D. I. Cohen, E. A. Nielsen, and M. M. Davis.** 1984. Isolation of cDNA clones encoding T cell-specific membrane-associated proteins. Nature (London) 308:149–153.
23. **Hedrick, S. M., I. Engel, D. L. McElligott, P. J. Fink, M. Hsu, D. Hansburg, and L. A. Matis.** 1988. Selection based on specificity for amino acid sequences in the putative third hypervariable region of the beta chain of the T cell antigen receptor. Science 239:1541–1544.
24. **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 (London) 326:307–308.
25. **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: primary structure of the antigen-MHC receptor of four hapten-specific cytotoxic T cell clones. J. Exp. Med. 165:591–600.
26. **Joly, E., M. Salvato, J. L. Whitton, and M. B. A. Oldstone.** 1989. Polymorphism of cytotoxic T-lymphocyte clones that recognize a defined nine-amino-acid immunodominant domain of lymphocytic choriomeningitis virus glycoprotein. 1989. J. Virol. 63:1845–1851.
27. **Kanagawa, O., E. Palmer, and J. Bill.** 1989. The T cell receptor $V_\beta 6$ domain imparts reactivity to the Mls-1^a antigen. Cell Immunol. 119:412–426.
28. **Land, H., M. Grez, H. Hauser, W. Lindermaier, and G. Schutz.** 1981. 5'-Terminal sequences of eucaryotic mRNA can be cloned with high efficiency. Nucleic Acids Res. 9:2251–2266.
29. **Lehmann-Grube, F.** 1971. Lymphocytic choriomeningitis virus. Virol. Monogr. 10:1–173.
30. **Loh, E. Y., J. F. Elliott, S. Cwirla, L. L. Lanier, and M. M. Davis.** 1989. A novel PCR procedure using single-sided specificity: analysis of T-cell receptor δ chain. Science 243:217–220.
31. **Nikolic-Zugic, J., and M. J. Bevan.** 1988. Thymocytes expressing CD8 differentiate into $CD4^+$ cells following intrathymic injection. Proc. Natl. Acad. Sci. USA 85:8633–8637.
32. **Oldstone, M. B. A., J. L. Whitton, H. Lewicki, and A. Tishon.** 1988. Fine dissection of a nine amino acid glycoprotein epitope, a major determinant recognized by lymphocytic choriomeningitis virus-specific class I-restricted H-$2D^b$ cytotoxic T lymphocytes. J. Exp. Med. 168:559–570.
33. **Parekh, B. S., and M. J. Buchmeier.** 1986. Proteins of lymphocytic choriomeningitis virus: antigenic topography of the viral glycoproteins. Virology 153:168–178.
34. **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 cytotoxic T cells and identification of a new V_α family. Eur. J. Immunol. 17:1843–1846.
35. **Riviere, Y., P. J. Southern, R. Ahmed, and M. B. A. Oldstone.** 1986. Biology of cloned cytotoxic T lymphocytes specific for lymphocytic choriomeningitis virus. V. Recognition is restricted to gene products encoded by the viral S RNA segment. J. Immunol. 136:304–307.
36. **Rodewald, H.-R., U. H. Koszinowski, K. Eichmann, and I. Melchers.** 1989. Predominant utilization of $V_\beta 8^+$ T cell receptor genes in the H-2 L^d-restricted cytotoxic T cell response against the immediate-early protein pp89 of the murine cytomegalovirus. J. Immunol. 143:4238–4243.
37. **Roehm, N., L. Herron, J. Cambier, D. DiGuisto, K. Haskins, J. Kappler, and P. Marrack.** 1984. The major histocompatibility complex-restricted antigen receptor on T cells: distribution of thymus and peripheral T cells. Cell 38:577–584.
38. **Rowe, W. P.** 1954. Studies on pathogenesis and immunity in lymphocytic choriomeningitis infection of the mouse. Research Report NM 005048.14.01. Naval Medical Research Institute, Bethesda, Md.
39. **Rupp, F., J. Brecher, M. A. Giedlin, T. Mosmann, R. M. Zinkernagel, H. Hengartner, and R. H. Joho.** 1987. T-cell antigen receptors with identical variable regions but different diversity and joining region segments have distinct specificities but cross-reactive idiotypes. Proc. Natl. Acad. Sci. USA 84:219–222.
40. **Saiki, R. K., D. H. Gelfand, S. Stoffel, S. J. Scharf, R. Higuchi, G. T. Horn, K. B. Mullis, and H. A. Erlich.** 1988. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science 239:487–491.
41. **Saito, H., D. M. Kranz, Y. Takagaki, A. C. Hayday, H. N. Eisen, and S. Tonegawa.** 1984. Complete primary structure of a heterodimeric T-cell receptor deduced from cDNA sequences. Nature (London) 309:757–762.
42. **Saito, H., D. M. Kranz, Y. Takagaki, A. C. Hayday, H. N. Eisen, and S. Tonegawa.** 1984. A third rearranged and expressed gene in a clone of cytotoxic T lymphocytes. Nature (London) 312:36–40.
43. **Sakai, K., A. A. Sinha, D. J. Mitchell, S. S. Zamvil, J. B. Rothbard, H. O. McDevitt, and L. Steinman.** 1988. Involvement of distinct murine T-cell receptors in the autoimmune encephalitogenic response to nested epitopes of myelin basic protein. Proc. Natl. Acad. Sci. USA 85:8608–8612.
44. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA 74:5463–5467.
45. **Schulz, M., P. Aichele, M. Vollenweider, F. W. Bobe, F. Cardinaux, H. Hengartner, and R. M. Zinkernagel.** 1989. Major histocompatibility complex-dependent T cell epitopes of lymphocytic choriomeningitis virus nucleoprotein and their protective capacity against viral disease. Eur. J. Immunol. 19:1657–1667.
46. **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 bm12 mutant mice. Mol. Cell. Biol. 7:1865–1872.
47. **Spinella, D. G., T. H. Hansen, W. D. Walsh, M. A. Behlke, J. P.**

Tillinghast, H. S. Chou, P. J. Whiteley, 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:**3991–3995.

48. **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:**3994–4000.
49. **Tan, K.-N., B. M. Batlof, J. A. Gilmore, A. C. Kronman, J. H. Lee, A. M. Maxam, and A. Rao.** 1988. The T cell receptor $V_\alpha3$ gene segment is associated with reactivity to p-azobenzenearsonate. Cell **54:**247–261.
50. **Townsend, A., and H. Bodmer.** 1989. Antigen recognition by class I-restricted T lymphocytes. Annu. Rev. Immunol. **7:**601–624.
51. **Urban, J. L., V. Kumar, D. H. Kono, C. Gomez, S. J. Horvath, J. Clayton, D. G. Ando, E. E. Sercarz, and L. Hood.** 1988. Restricted use of T cell receptor V genes in murine autoimmune encephalomyelitis raises possibilities for antibody therapy. Cell **54:**577–592.
52. **Whitton, J. L., J. R. Gebhard, H. Lewicki, A. Tishon, and M. B. A. Oldstone.** 1988. Molecular definition of a major cytotoxic T-lymphocyte epitope in the glycoprotein of lymphocytic choriomeningitis virus. J. Virol. **62:**687–695.
53. **Whitton, J. L., A. Tishon, H. Lewicki, J. Gebhard, T. Cook, M. Salvato, E. Joly, and M. B. A. Oldstone.** 1989. Molecular analyses of a five-amino-acid cytotoxic T-lymphocyte (CTL) epitope: an immunodominant region which induces nonreciprocal CTL cross-reactivity. J. Virol. **63:**4303–4310.
54. **Winoto, A., J. L. Urban, N. C. Lan, J. Goverman, L. Hood, and D. Hansburg.** 1986. Predominant use of a V_α gene segment in mouse T-cell receptors for cytochrome C. Nature (London) **324:**679–682.
55. **Wysocki, L. J., and V. L. Sato.** 1978. "Panning" for lymphocytes: a method for cell selection. Proc. Natl. Acad. Sci. USA **75:**2844–2848.
56. **Yanagi, Y., Y. Yoshikai, K. Leggett, S. P. Clark, I. Aleksander, and T. W. Mak.** 1984. A human T cell-specific cDNA clone encodes a protein having extensive homology to immunoglobulin chains. Nature (London) **308:**145–149.
57. **Zinkernagel, R. M., and P. C. Doherty.** 1974. Restriction of in vitro T cell mediated cytotoxicity in lymphocytic choriomeningitis within a syngeneic or semiallogeneic system. Nature (London) **248:**701–702.
58. **Zinkernagel, R. M., and P. C. Doherty.** 1979. MHC-restricted cytotoxic T cells: studies on the biological role of polymorphic major transplantation antigens determining T-cell restriction-specificity, function and responsiveness. Adv. Immunol. **27:**51–177.