T-Cell Receptors from Virus-Specific Cytotoxic T Lymphocytes Recognizing a Single Immunodominant Nine-Amino-Acid Viral Epitope Show Marked Diversity

MARC S. HORWITZ,* YUSUKE YANAGI, AND MICHAEL B. A. OLDSTONE

Division of Virology, Department of Neuropharmacology, The Scripps Research Institute, La Jolla, California 92037

Received 6 August 1993/Accepted 8 October 1993

Following infection of the *H-2^d* mouse by lymphocytic choriomeningitis virus, the newly generated cytotoxic T lymphocyte (CTL) response is focused to a single 9-amino-acid peptide sequence (epitope) of the virus. More than 96% of the primary, secondary, and clonal CTL respond to this lymphocytic choriomeningitis virus nucleoprotein epitope. This unique system affords the opportunity to evaluate the T-cell response to a single viral CTL epitope in a case in which the outcome of infection, either viral clearance or host death, is mediated by the CTLs. Specifically, the molecular structure of the T-cell receptors (TCRs) of CTLs responding to this epitope was analyzed. By using an anchored polymerase chain reaction, the TCR chains of three CTL clones cDNAs were amplified, sequenced, and found to have unique V_{α} or V_{β} chains relative to each other as well as to lack restriction to any particular variable chain. These data indicate that the highly diverse antiviral CTL response is pleomorphic and probably provides an advantage to the host as it limits the emergence of viral variants that could more easily arise if the TCR response were homogeneous.

In response to a viral infection, the host relies on its immune system for control and elimination of the virus. Central to this immune response is the ability of T cells to recognize foreign antigens as either degraded or processed peptides in the context of self major histocompatibility complex (MHC) molecules (4, 10). For the most part, cytotoxic T lymphocytes (CTLs) recognize antigen presented by class I MHC molecules, whereas T helper cells recognize antigens presented by class II MHC molecules. T-cell recognition of antigen is carried out by the T-cell receptor (TCR), a membrane bound heterodimer composed of α and β chains (10). Recognition of the viral antigen-MHC complex by the TCR results in destruction of the infected cell by the virus-specific CTL. Similar to immunoglobulins, TCRs are encoded by a series of rearranged gene segments noted as variable (V), diversity (D), joining (J), and constant (C) (9, 40). TCR diversity is generated by at least four mechanisms: rearrangement of the V, D, and J gene segments; imprecise joining at these sites; random addition of nucleotides between these segments during recombination; and random pairing of the α and β chains that form the heterodimer (36). The TCR is structurally similar to the immunoglobulins, and its antigen-binding loops or complementarity-determining regions (CDRs) have been predicted to have a similar location and conformation (8). Within the structure of the TCR molecule, a significant degree of diversity has been concentrated in the third CDR loop of both chains, which is encoded by the V(D)J junction, and a compelling argument has been made that this loop is responsible for recognition of the peptide-MHC complex (8, 10, 18).

Dominant or restricted usage of a particular V gene segment has been observed for both CTLs and T helper cells in response to a number of specific antigens from both viral (3, 32, 42) and nonviral (1, 6, 11, 14, 24, 34, 35, 41) sources. The presence of a limited TCR repertoire specific for antigens

central to an immunologically mediated event allows for intervention in the immune response with TCR-specific antibodies. This approach has successfully been used to prevent the development of autoimmune disease-mediated pathogenesis both by the addition of exogenously generated antibodies (1, 35) and by the induction of the endogenous humoral response to a specific TCR chain through vaccination (17).

The T-cell response of mice to lymphocytic choriomeningitis virus (LCMV) provides an excellent model with which to study the structure of the TCR during virus-induced immunopathology. In mice, differences in the route of infection by LCMV can lead to either viral clearance or death (5); both events are mediated by the LCMV-specific CTLs of the mouse (44). T-cell epitopes and presenting MHC molecules have been well characterized in the LCMV-specific CTL response (25, 28, 37, 38). In BALB/C mice (*H-2^d*), more than 96% of the LCMV-specific CTL response at both the bulk population and clonal level is restricted to a single 9-amino-acid (aa) epitope from the viral nucleoprotein (NP, aa 119 to 127; PQASGVYMG) presented by the *L^d* class I MHC molecule (38).

In this paper, we report the analysis of the TCR structure from CTL clones derived from *H-2^d* responders having specificity for this predominant NP epitope and asked whether the TCRs of the CTLs responding to LCMV NP have a limited V_{α} and/or V_{β} response. We find a diverse TCR response to this immunodominant epitope, discuss the likely implications of a nonhomogeneous TCR response, and address why such a virus-mediated immune response would differ from those reported for "self" antigens.

MATERIALS AND METHODS

CTL clones. Isolation and maintenance of LCMV-specific *L^d* (*H-2^d*) CTL clones used in this study were described previously (38). The Armstrong CA1371 strain clone 53b (ARM) of LCMV was used for their generation and maintenance.

RNA and anchored PCR. Cytoplasmic RNA was extracted from CTL clones by using Nonidet P-40-sodium dodecyl

* Corresponding author. Mailing address: Division of Virology, Department of Neuropharmacology, The Scripps Research Institute, 10666 N. Torrey Pines Rd., La Jolla, CA 92037. Phone: (619) 554-9100. Fax: (619) 554-9981.

sulfate (SDS)-phenol. Total CTL RNA was used as substrate in a modified anchored polymerase chain reaction (PCR) (23, 42). First-strand cDNA was synthesized from total cytoplasmic RNA by using an oligo(dT) primer and reverse transcriptase. The RNA template was degraded with RNase H. cDNA was separated from free nucleotides and primer by using gel electrophoresis or GlassMax spin cartridges (GIBCO-BRL, Gaithersburg, Md.). A poly(dG) or poly(dC) tail was then added to the 3' end of the cDNA with terminal deoxytransferase and either dGTP or dCTP. Tailed cDNA was amplified in a PCR with either C_α or C_β primer plus an anchor primer which could anneal to the 3' poly(dG) or poly(dC) tail. The β chains were targeted and amplified by the modified anchored PCR approach described above and cloned directly by using the TA cloning kit (Invitrogen, San Diego, Calif.). The α chains were targeted and amplified by the 5' RACE system (GIBCO-BRL) and cloned into pAMP1 by using the Clone-Amp system (GIBCO-BRL). Colonies obtained by both methods were screened by growing isolated single colonies overnight in 0.1 ml of LB medium plus appropriate antibiotic followed by placing 10 μ l of the bacterial culture directly into a 50- μ l PCR with the primers described below.

Oligonucleotides. Oligonucleotide primers were synthesized at The Scripps Research Institute Microchemical Core facility. C_α (GACCGAGGATCTTTAACTGGTACAC) and C_β (GGTGGAGTCACATTTCTCAGATCCTC) were derived from the known sequences of the murine constant region genes and used for the anchor PCR amplification. Additional nested primers, C_α seq (GAGCTGTCCTGAGACCGAGG) and C_β seq (ATCTCTGCTTTTGATGGCTC), as well as universal and reverse M13 primers, were used for the screening of colonies by PCR and subsequent sequencing of these clones.

Sequence and computer analysis. The nucleotide sequences of the cloned PCR products were determined by the dideoxy method of Sanger et al. (33) adapted for double-stranded templates and with T7 DNA polymerase. Sequence data were analyzed by using a Sun computer equipped with IntelliGenetics programs including FASTDB, SEQ, and PEP.

Antibodies and immunofluorescence staining. Murine TCR V_β -specific primary antibodies used in these experiments were MR10-2 (V_β 9, mouse immunoglobulin G [IgG]) (42) and B21.5 (V_β 10, rat IgG) (Pharmingen, La Jolla, Calif.). Secondary antibodies used were mouse IgG antibody labeled with fluorescein isothiocyanate and rat IgG antibody labeled with fluorescein isothiocyanate. Immunofluorescence staining was carried out on 5×10^5 cells that were incubated at 4°C for 30 min with a primary antibody. Following washing, the cells were incubated with the appropriate FITC-labeled antibody at 4°C for 30 min, washed three times, and resuspended in 1% paraformaldehyde. Cells were analyzed on a FACScan flow cytometer (Becton Dickinson).

Nucleotide sequence accession numbers. Sequence data for the three CTLs and six rearranged TCR genes have been entered in the GenBank data base under the following accession numbers: L16801 (HD3 V_α), L16802 (HD3 V_β), L16803 (HD8 V_α), L16804 (HD8 V_β), L16799 (HD47 V_α), and L16800 (HD47 V_β).

RESULTS

Molecular analysis of TCRs from LCMV-specific CTL clones in the BALB/c mouse. To examine the structure of the TCR involved in the recognition of LCMV-infected $H-2^d$ targets, we used three LCMV-specific CTL clones, HD3, HD8, and HD47, for sequence analysis. All three clones recognize the NP epitope (aa 119 to 127) of LCMV ARM presented by

TABLE 1. TCR sequences of LCMV-specific CTL clones

CTL clone	α -Chain sequence	β -Chain sequence
HD8	$V_{\alpha 1} J_{\alpha TA13} C_\alpha$	$V_{\beta 10.1} D_\beta J_{\beta 2.5} C_{\beta 2}$
HD47	$V_{\alpha 3} J_{\alpha 3} C_\alpha$	$V_{\beta 6} D_\beta J_{\beta 2.2} C_{\beta 2}$
HD3	$V_{\alpha 2} J_{\alpha 3} C_\alpha$	$V_{\beta 7} D_\beta J_{\beta 2.6} C_{\beta 2}$

L^d (38). However, each clone recognizes peptides of distinct lengths and minor variations in the amino acid patterns within this epitope restricted by the L^d component of $H-2^d$ (38; data not shown). Precisely, HD47 can recognize the analogous peptide from the Pasteur strain of LCMV (LKTSGVYMG), whereas HD3 and HD8 recognize only the ARM sequence (PQASGVYMG). Although HD8 and HD47 can recognize a minimal 6-aa (SGVYMG) peptide efficiently, a 5-aa peptide (GVYMG) is all that HD3 requires. All three CTL clones have in vitro and in vivo effector function (21, 38; data not shown). RNA was extracted from each CTL clone and used as target for anchored PCR amplification of both α and β TCR chains. Primers were derived from the constant regions of both α and β chains. PCR products were cloned and subjected to sequence analysis. At least three independent cDNA clones from each rearranged TCR chain gene were studied.

Only a single mRNA with a complete open reading frame was observed per chain from each of the three CTL clones. Table 1 summarizes the results and indicates that three different V_α ($V_{\alpha 1}$, $V_{\alpha 2}$, and $V_{\alpha 3}$) and three different V_β ($V_{\beta 6}$, $V_{\beta 7}$, and $V_{\beta 10.1}$) gene segments made up the three different CTL clones. Thus, no common V gene segment for α or β was found for the three CTL clones analyzed. Two clones, HD3 and HD47, used the same J_α segment; however, none of the J_β segments were shared. To further document that the TCR sequences expressed at the RNA level were present on the cell surface, monoclonal antibody specific to $V_{\beta 10}$ was shown to stain $V_{\beta 10}$ antigen on the surface of the CTL clone HD8 (Fig. 1), thereby linking the data accumulated by PCR analysis to the expression of the cell surface molecule. The slight increase observed in fluorescence with $V_{\beta 9}$ represents nonspecific background due to the fluorescein isothiocyanate-labeled goat antibody to mouse IgG and was seen in repeated experiments. Comparisons of sequence similarity of these recombined TCR genes with the known TCR sequences showed that all three V_β gene segments were identical to the previously reported sequences (19, 20). V_α gene segments of CTL clones HD3, HD8, and HD47 were 95 to 100% similar to known V_α families (7, 15, 30). Differences noted were probably due to V_α chain family or strain diversity and had minimal impact on the predicted amino acid sequence.

Sequence comparisons of the three TCRs which recognize the same antigenic peptide are presented in Fig. 2 and 3. Like Igs, the TCR maintains CDRs (8, 10). The CDR-3-like domain of the TCR is believed to be responsible for antigen recognition and corresponds to the V(D)J region of the TCR, specifically, across the junctional regions of the V_α - J_α , V_β - D_β , and D_β - J_β portions. This region includes the N-region additions and corresponds to only a small portion of the area encoded by the 3' portion of the V gene and segments 5' portion of the J gene segments. When we compared sequences across the CDR-3-like domains of each chain of the three CTL clones, we noted no obvious amino acid pattern that might be associated with antigen recognition. However, similar amino acids across both the α and β chains were identified (see underlined regions in Fig. 2 and 3), suggesting that there were

FIG. 1. Cell surface expression of the $V_{\beta}10$ β chain on the LCMV-specific CTL clone HD8. Cells were stained with either anti- $V_{\beta}9$ or anti- $V_{\beta}10$ antibodies as described in Materials and Methods. A total of 5,000 cells were analyzed for each sample.

similar patterns which link the β -chain CDR-3-like domains (GGA, TGQ). We also observed the presence of amide-containing amino acids (asparagine [N] or glutamine [Q]) across this domain in all six chains; specifically, asparagine residues are present in all three α chain CDR-3-like domains. There was no similarity in the length of the domains, nor were aromatic residues consistently present across either the α or β CDR-3-like domains. Additionally, despite the use of the same J_{α} segment, HD3 and HD47 do not possess similarity across their respective CDR-3-like domains other than that provided by the J segment.

DISCUSSION

Three independent and distinct CTL clones specific for the predominant T-cell epitope of LCMV (NP aa 119 to 127; PQASGVYMG) presented by the L^d MHC molecule are not homogeneous for any V_{α} or β chain (Table 1). A single J_{α} segment was shared by two of the CTLs; however, the V-J joining regions encoding the CDR-3-like domain were not similar in sequence or structure. Thus, we found no evidence of a restricted V_{α} or V_{β} TCR response made to LCMV in $H-2^d$ mice.

The highly diverse TCR response accompanying LCMV infection most probably provides a selective advantage to the host. Because of TCR diversity, the ability of the virus to generate CTL escape variants is decreased by simply increasing the number of conformational possibilities which recognize a single viral peptide-MHC complex. At present, CTL escape variants have only been noted during in vitro selection experiments with LCMV (2, 22) and in a stringent in vivo study in which LCMV-specific TCR transgenic mice were used (31). Changes in CTL epitopes during natural human immunodeficiency

FIG. 2. Comparison of the nucleotide and predicted amino acid sequences that form the VJ junctional region of the TCR α chain from three LCMV-specific CTL clones. Similar amino acids between at least two of the three TCR chains for the α chain are underlined. The GenBank accession numbers for the TCR sequences are L16803 for HD8, L16799 for HD47, and L16801 for HD3.

TCR

HD 8

V β 10.1	D β + N	J β 2.5	C β 2
TGTGCCAGCAGC	TCTCGGACTGGGCAAGACACCCAGTACTTTGGGCCAGGCACTCGGCTCCTCGTGTAGAGGATCTG		
<u>C A S S</u>	<u>S R T G Q D T Q Y F G P G T R L L V L E D L</u>		

HD 47

V β 6	D β + N	J β 2.2	C β 2
TGTGCCAGCAGTATACCGGGGGCGCGGACACCGGGCAGCTCTACTTTGGTGAAGGCTCAAAGCTGACAGTGCTGGAGGATCTG			
<u>C A S S</u> I P <u>G G A N T G Q L Y F G E G S K L T V L E D L</u>			

HD 3

V β 7	D β + N	J β 2.6	C β 2
TGTGCTAGCAGT	TTCGGGGGGGCTGGCGAACAGTACTTCGGTCCCGGCACCAGGCTCACGGTTTTAGAGGATCTG		
<u>C A S S</u>	<u>F G G A G E Q Y F G P G T R L T V L E D L</u>		

FIG. 3. Comparison of the nucleotide and predicted amino acid sequences that form the VDJ junctional region of the TCR β chain from three LCMV-specific CTL clones. Similar amino acids between at least two of the three TCR chains for the β chain are underlined. The GenBank accession numbers for the TCR sequences are L16804 for HD8, L16800 for HD47, and L16802 for HD3.

ciency virus type 1 infection, but not CTL escape variants, were reported by Phillips et al. (29).

The precise molecular characterization of several TCRs recognizing the LCMV NP aa 119 to 126 presented by the L^d MHC molecule is given in Fig. 2 and 3. The CDR-1 and CDR-2-like domains of the TCR encoded on the V gene segments are probably responsible for MHC recognition, whereas the CDR-3-like regions encoded by the recombination junction of the rearranged gene are responsible for recognition of the MHC-presented peptide fragment (8, 10). The lack of homogeneity to any one V α or V β chain suggests that although the CDR-1 and CDR-2-like regions recognize L^d , there is no preferential binding of any particular V chain to the MHC molecule. The predicted CDR-3-like domains show no obvious amino acid pattern that can be associated with antigen recognition.

Despite the presence of a few shared residues, the generation of additional data with other viral peptide sequences, including the use of site-specific mutants in the viral sequences and responding TCR structures and crystallographic analysis, will be required before a precise molecular analysis of the TCR-antigen-MHC complex can be made. With this in mind, we have been generating viral CTL escape mutants and testing the altered epitope sequences as peptides for their ability to bind MHC molecules as well as to be recognized by CTLs. Additionally, viral peptides representing the LCMV epitopes restricted by L^d and D^b have been made with single-amino-acid substitutions and are currently under study (16).

Earlier work indicated a restriction of CTLs specific for the GP2 epitope (glycoprotein [GP] aa 276 to 286; SGVENPG GYCL) of LCMV ARM and WE presented by the D^b MHC molecule of $H-2^b$ mice to the TCR chain V α 4 (3, 42). However,

when CTLs specific for the two other CTL epitopes presented by D^b LCMV ARM NP (aa 396 to 404, FQPNGQFI) and GP1 (aa 33 to 42, KAVYNFATCG) were studied (43), no restriction to the V α 4 TCR subfamily was observed. Likewise, in this study we did not find V α 4 to be used by any of the LCMV NP (aa 119 to 126)-specific $H-2^d$ -restricted CTLs analyzed. Thus, there are now sufficient data to obtain a picture of the specificity of the immune response to a single virus in which four epitopes are presented across two MHC backgrounds ($H-2^b$ and $H-2^d$). Although the response to a single epitope (GP aa 276 to 286, D^b) is restricted to V α 4, responses to the other three CTL epitopes show no restriction to any specific TCRs including V α 4. Interestingly, there is no TCR restriction to the predominant L^d presented epitope, LCMV NP (aa 119 to 126), despite the observation that more than 96% of the CTLs responding at both the bulk population and clonal levels recognize this single 9-aa epitope (38). Additionally, the observed lack of homogeneity among the NP-specific L^d -restricted CTLs reduces the possibility of selection during the generation of these CTL clones.

There is another consequences of a diverse TCR response. CTLs destroy virally infected cells that present appropriate viral peptide complexed with class I MHC glycoprotein. In some instances it is beneficial to modulate and dampen the immune response, especially when the resultant CTL-mediated injury results in more risk than benefit to the host (e.g., during encephalitis, leptomenigitis, or myocarditis) or acts to induce or support long-term autoimmune disease (26, 39). Of the several strategies envisioned, our data indicate that it is highly unlikely that therapy directed at blocking the TCR (i.e., by use of antibody against a specific variable chain) for preventing virus-induced immunopathology would be success-

ful. Since humans have a more complex pattern of MHC presentation than do inbred mice, TCR-directed immunotherapy in viral infections in humans is not likely to be a useful approach.

The enormous diversity available within the T-cell response should predictably lead to a lack of homogeneity in the TCR response to the whole virus or a given viral epitope. Why, then, do homogeneous TCR responses occur against several reported foreign T-cell epitopes (1, 3, 6, 11, 14, 24, 32, 34, 35, 41, 42)? Foreign T-cell epitopes can be subdivided into two groups, "self"-like and non-"self"-like. TCR homogeneity has been observed primarily in the self-like group, which includes cross-species molecules such as whale myoglobin, pigeon cytochrome *c*, human leukocyte antigen molecules, and some viral gene products that probably evolved from or evolved to mimic host protein structure or function. Natural tolerance to self peptides presented by class I MHC molecules substantially reduces the size of the TCR repertoire of CTLs that respond to antigenic (viral) peptides homologous to self (6) and thus controls T-cell diversity. The remaining T cells would not be a highly variable subset, possibly even derived from the same V gene families, and this association to the same V gene family may imply that the limited V gene sequences are responsible for less efficient recognition of native peptide in the context of MHC. Although T cells escaping deletion might not mount an efficient response to self antigen *in vivo*, a slightly modified self-like antigen from an external (e.g., viral) source may be recognized more efficiently and lead to the amplification of this population of restricted T cells. To test this hypothesis, we have identified a putative self peptide similar to a viral CTL epitope (LCMV GP2, aa 278 to 286), which elicits a restricted V α 4 TCR response (42) and are currently addressing whether this self peptide can be presented like its viral counterpart. These findings have implications for several autoimmune diseases in which a preponderance of TCR homogeneity to self epitopes occurs. Limited TCR repertoires have been observed in experimental models of human autoimmune disease (experimental autoimmune encephalomyelitis, adjuvant arthritis, and experimental autoimmune thyroiditis) and in each case were associated with T-cell reactivity to self antigen (12). These leads are currently being followed in humans and experimental animals undergoing demyelination after acute viral infection or vaccination (13, 26, 27).

ACKNOWLEDGMENTS

This work was supported in part by Public Health Service grants AI-09484 and NS-12428 by and postdoctoral training grant MH-19185 (to M.S.H.) from the National Institutes of Health.

We thank H. Lewicki and J. Arcediano for generation and maintenance of the CTL clones, B. Cubitt for technical assistance, and C. F. Evans, G. F. Rall, and J. L. Whitton for reviewing the manuscript.

REFERENCES

- Acha-Orbea, H., D. Mitchell, L. Timmermann, D. Wraith, G. Tausch, M. Waldor, S. Zamvil, H. McDevitt, and L. Steinman. 1988. Limited heterogeneity of T cell receptors from lymphocytes mediating autoimmune encephalomyelitis allows specific immune intervention. *Cell* **54**:263–273.
- Aebischer, T., D. Moskopidid, U. Rohrer, R. Zinkernagel, and H. Hengartner. 1991. *In vitro* selection of lymphocytic choriomeningitis virus escape mutants by cytotoxic T lymphocytes. *Proc. Natl. Acad. Sci. USA* **88**:11047–11051.
- Aebischer, T., S. Oehen, and H. Hengartner. 1990. Preferential usage of V α 4 and V β 10 T cell receptor genes by LCMV glycoprotein specific H-2D^b restricted cytotoxic T cells. *Eur. J. Immunol.* **20**:523–531.
- Bjorkman, P., and P. Parham. 1990. Structure, function, and diversity of class I major histocompatibility complex molecules. *Annu. Rev. Biochem.* **59**:253–288.
- Buchmeier, M., R. Welsh, F. Dutko, and M. B. A. Oldstone. 1980. The virology and immunobiology of lymphocytic choriomeningitis virus infection. *Adv. Immunol.* **30**:275–331.
- Casanova, J. L., J. C. Cerottini, M. Matthes, A. Necker, H. Gournier, C. Barra, C. Widmann, H. R. MacDonald, F. Lemonnier, B. Malissen, and J. L. Maryanski. 1992. H-2 restricted cytolytic T lymphocytes specific for HLA display T cell receptors of limited diversity. *J. Exp. Med.* **176**:439–447.
- Chien, Y., D. Becker, T. Lindsten, M. Okamura, D. Cohen, and M. Davis. 1984. A third type of murine T-cell receptor. *Nature (London)* **312**:31–35.
- Clothia, C., D. Boswell, and A. Lesk. 1988. The outline structure of the T-cell $\alpha\beta$ receptor. *EMBO J.* **7**:3745–3755.
- Davis, M. 1990. T cell receptor gene diversity and selection. *Annu. Rev. Biochem.* **59**:475–496.
- Davis, M., and P. Bjorkman. 1988. T cell antigen receptor genes and T cell recognition. *Nature (London)* **334**:395–402.
- Engel, I., and S. 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**:473–484.
- Esch, T., L. Clark, X. Zhang, S. Goldman, and E. Heber-Katz. 1992. Observations, legends, and conjectures concerning restricted T-cell receptor usage and autoimmune disease. *Crit. Rev. Immunol.* **11**:249–264.
- Fenner, F., B. R. McAuslan, C. A. Mims, J. Sambrook, and D. O. White. 1974. The biology of animal viruses, p. 545–585. Academic Press, Inc., New York.
- 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.
- Fondell, J., J. P. Marolleau, D. Primi, and K. Marcu. 1990. On the mechanism of non-allelically excluded Va-Ja T cell receptor secondary rearrangements in a murine T cell lymphoma. *J. Immunol.* **144**:1094–1103.
- Gairin, J. E., and M. B. A. Oldstone. 1993. Fine analysis and comparison of the optimal MHC binding sequence of the H-2Db epitopes of lymphocytic choriomeningitis virus. *J. Cell. Biochem.* **17D**:105.
- Gaur, A., R. Haspel, J. Mayer, and C. G. Fathman. 1993. Requirement for CD8⁺ cells in T cell receptor peptide-induced clonal unresponsiveness. *Science* **259**:91–94.
- Goverman, J., K. Minard, N. Shastri, T. Hunkapiller, D. Hansburg, E. Sercarz, and L. Hood. 1985. Rearranged β T cell receptor genes in a helper T cell clone specific for lysozyme: no correlation between V β and MHC restriction. *Cell* **40**:859–867.
- Hirama, T., S. Takeshita, Y. Matsubayashi, M. Iwashiro, I. Masuda, K. Kuribayashi, Y. Yoshida, and H. Yamagishi. 1991. Conserved V(D)J junctional sequence of cross-reactive cytotoxic T cell receptor idotype and the effect of a single amino acid substitution. *Eur. J. Immunol.* **21**:483–488.
- Iwamoto, A., P. Ohashi, H. Pircher, C. Walker, 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**:591–600.
- Klavinskis, L., A. Tishon, and M. B. A. Oldstone. 1989. Efficiency and effectiveness of cloned virus-specific cytotoxic T lymphocytes *in vivo*. *J. Immunol.* **143**:2013–2016.
- Lewicki, H., A. Tishon, C. Evans, P. Borrow, and M. B. A. Oldstone. Generation and characterization of CTL escape variants during LCMV infection. Submitted for publication.
- Loh, E., J. Elliott, S. Cwirla, L. Lanier, and M. Davis. 1989. Polymerase chain reaction with single-sided specificity: analysis of T cell receptor δ chain. *Science* **243**:217–220.
- Morel, P., A. Livingstone, and C. Fathman. 1987. Correlation of T cell receptor V β gene family with MHC restriction. *J. Exp. Med.* **166**:583–588.
- Oldstone, M., J. Whitton, H. Lewicki, and A. Tishon. 1988. Fine dissection of a nine amino acid glycoprotein epitope, a major determinant recognized by LCMV-specific class I-restricted H-2Db cytotoxic T lymphocytes. *J. Exp. Med.* **168**:559–570.
- Oldstone, M. B. A. 1989. Infectious agents as etiologic triggers of

- autoimmune disease. *Curr. Top. Microbiol. Immunol.* **145**:127–135.
27. **Oldstone, M. B. A.** 1987. Molecular mimicry and autoimmune disease. *Cell* **50**:819–820.
 28. **Orn, A., R. Goodenow, L. Hood, P. Brayton, J. Woodward, R. Harmon, and J. Frelinger.** 1982. Product of a transferred H-2Ld gene acts as restriction element for LCMV-specific killer T cells. *Nature (London)* **297**:415–417.
 29. **Phillips, R., S. Rowland-Jones, D. Nixon, F. Gotch, J. Edwards, A. Ogunlesi, J. Elvin, J. Rothbard, C. Bangham, C. Rizza, and A. McMichael.** 1991. Human immunodeficiency virus genetic variation that can escape cytotoxic T cell recognition. *Nature (London)* **354**:453.
 30. **Pircher, H., E. E. Michalopoulos, A. Iwamoto, P. S. Ohasi, 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 (alpha) family. *Eur. J. Immunol.* **17**:1843–1846.
 31. **Pircher, H., D. Moskophidis, U. Rohrer, K. Burki, H. Hengartner, and R. Zinkernagel.** 1990. Viral escape by selection of cytotoxic T cell-resistant virus variants in vivo. *Nature (London)* **346**:629.
 32. **Rodewald, H.-R., U. Koszinowski, K. Eichmann, and I. Melchers.** 1989. Predominant utilization of V β 8 T cell receptor genes in the H-2L^d restricted cytotoxic T cell response against the immediate early protein pp89 of the murine cytomegalovirus. *J. Immunol.* **143**:4238–4243.
 33. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. *Proc. Natl. Acad. Sci. USA* **74**:5463–5467.
 34. **Tan, K.-N., B. Datlof, J. Gilmore, A. Kronman, J. Lee, A. Maxam, and A. Rao.** 1988. The T cell receptor V α 3 gene segment is associated with reactivity to *p*-azobenzene arsonate. *Cell* **54**:247–261.
 35. **Urban, J., V. Kumar, D. Kono, C. Gomez, S. Horvath, J. Clayton, D. Ando, 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.
 36. **Usuku, K., N. Joshi, and S. L. Hauser.** 1992. T-cell receptors: germline polymorphism and patterns of usage in demyelinating diseases. *Crit. Rev. Immunol.* **11**:381–393.
 37. **Whitton, J., J. Gebhard, H. Lewicki, A. Tishon, and M. Oldstone.** 1988. Molecular definition of a major cytotoxic T-lymphocyte epitope in the glycoprotein of LCMV. *J. Virol.* **62**:687–695.
 38. **Whitton, J., A. Tishon, H. Lewicki, J. Gebhard, T. Cook, M. Salvato, E. Joly, and M. 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.
 39. **Whitton, J. L., and M. B. A. Oldstone.** 1990. Virus-induced immune response interactions: principles of immunity and immunopathology, p. 369–382. *In* B. N. Fields, D. M. Knipe, R. M. Chanock, M. S. Hirsch, J. L. Melnick, T. P. Monath, and B. Roizman (ed.), *Virology*, 2nd ed. Raven Press, New York.
 40. **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.
 41. **Winoto, A., J. Urban, N. Lan, J. Gorman, 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.
 42. **Yanagi, Y., R. Maekawa, T. Cook, O. Kanagawa, and M. Oldstone.** 1990. Restricted V-segment usage in T-cell receptors from cytotoxic T lymphocytes specific for a major epitope of LCMV. *J. Virol.* **64**:5919–5926.
 43. **Yanagi, Y., A. Tishon, H. Lewicki, B. Cubitt, and M. Oldstone.** 1992. Diversity of T-cell receptors in virus-specific cytotoxic T lymphocytes recognizing three distinct epitopes restricted by a single major histocompatibility complex molecule. *J. Virol.* **66**:2527–2531.
 44. **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.