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

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Before lysing an infected cell, CTL must first recognize a discrete viral protein sequence in the context of a restricting protein of the MHC on the cell (1). CTL play a significant role in terminating viral infection (2–9), and the inability to generate them can alter the outcome of viral invasion from an acute, self-limiting infection to a persistent, continuous one (10). Adoptive reconstitution of persistently infected hosts with previously primed virus-specific H-2-restricted CTL terminates the otherwise life-long infection (11). Yet, despite the role of CTL in influencing the outcome of infection, except primarily for studies with influenza virus (12–16), relatively little is known about the precise protein sequence(s) that CTL recognize, especially glycoproteins, and their relationship to restriction elements within the MHC.

To define the molecules involved in CTL recognition, we chose the model of lymphocytic choriomeningitis virus (LCMV)[1] in mice for several reasons. LCMV is a natural pathogen of mice (17), and the genetics of the mouse MHC is well understood and easily manipulated (18). CTL specific for LCMV are readily obtained and constitute a major immune response in vivo for terminating acute infection (2, 5, 17). Their absence is associated with persistent infection (1, 2, 11). Hence, data obtained from in vitro experimentation can be tested in vivo within a natural host-virus model.

LCMV has two RNA segments, a large (L) one of ~7.5 kb and a short (S) segment of 3.3 kb; CTL induction and recognition map to proteins encoded by genes on the S RNA (19, 20). The S RNA of LCMV Armstrong (ARM) encodes a 498 amino acid (aa) glycoprotein (GP) from its 5' ORF that is posttranslationally cleaved at residues 262–263 to yield GP-1 (aa 1–262) and GP-2 (aa 263–498) and a 558-aa nucleoprotein (NP) made from the 3' ORF (21,22). Earlier studies using cDNAs of LCMV GP or LCMV NP expressed in vaccinia virus (23, 24) indicated that the

H-2-restricting haplotype selected different LCMV proteins for CTL recognition epitopes and that the GP in H-2^b mice induced a prominent ($\sim$33% of the total) CTL response (23). Further dissection of the GP recognition sites in H-2^b (C57BL/6) mice using a series of LCMV ARM GP cDNA deletions from the 3' end indicated that the vast majority of H-2^b LCMV CTL clones (17 of 18: 94%) recognized GP, and 1 (6%) NP (23, 24). Further, the majority of GP CTL clones, 15/17 (88%), recognized targets expressing GP aa 272-293 (Table I). Here we report using five of these CTL clones, 232, 228, 31, RG-9, and 39, a battery of truncated peptides from GP aa 272-293 region, and site-specific amino acid substitutions to precisely identify and map the fine structure of the CTL epitope.

Materials and Methods

Target Cells, Viruses, and Mouse Strains.   BALB/c clone (Cl) 7 (K^{d}I^{d}D^d), B10.3R (K^{b}I^{b}D^d), B10.4R (K^{k}I^{k}D^b), and B10.5R (K^{b}I^{b}D^d) cells were grown in MEM supplement with 10% heat-inactivated FCS, 10^4 U of penicillin/ml, 10 mg of streptomycin/ml, and 1 mM L-glutamine, while MC57 (K^{b}I^{b}D^b) cells were grown in RPMI 1640 supplemented as above. Viruses used have been described previously (11, 23, 24) and consisted of LCMV ARM clone 53b, recombinant vaccinia virus:LCMV GP cDNA vectors expressing LCMV GP aa 1-363, 1-293, 1-271, 1-218, and vaccinia virus:LCMV NP cDNA vectors expressing LCMV NP 1-558. BALB/WEHI (W) (H-2^d) and C57BL/6 (H-2^b) mice were obtained from the closed vivarium breeding colony at the Research Institute of Scripps Clinic.

CTL Clones.   Specific CTL clones (H-2^b) were generated from C57BL/6 mice and maintained in culture as previously detailed (24). CTL clones were virus specific in that they failed to lyse target cells infected with vaccinia virus alone and H-2 restricted as they lysed H-2^b but not H-2^d targets infected with LCMV. The specificity of the clones used for experimentation are shown in Table I. CTL clones were used in E/T ratios of 5:1 and 2.5:1.

Primary Day 6-7 Splenic CTL.   LCMV-specific CTL were induced by intraperitoneal injection of 2 × 10^5 PFU of virus. Spleens were harvested 6-7 d after inoculation. Primary vaccinia CTL were induced by intraperitoneal inoculation of 2 × 10^6 PFU of virus, with harvesting on the 6th day. Single cell suspensions, free of erythrocytes, were used at E/T ratios of 50:1 and 25:1 (11, 24).

Cytotoxic Assays.   Killing by CTL was quantitated using a 4-5-h ^{51}Cr-release assay as described (11, 24). Target cell numbers used per assay point were 2 × 10^4. Samples were processed in triplicate and standard deviation among triplicates was <7%.

Peptide Synthesis.   Peptides were synthesized by the solid phase method described by Merrifield (25) with an automated peptide synthesizer (model 430A; Applied Biosystems, Inc., Foster City, CA) or by the tea bag method of Houghten (26). Peptides were cleaved from their insoluble polystyrene resin beads by hydrogen fluoride, extracted, lyophilized, and analyzed for purity by HPLC. Unless stated otherwise in the text, peptide, solubilized in RPMI, was incubated at a final concentration of 200 µg/ml with uninfected target cells throughout the 4-5-h period of the cytotoxic assay.

Results

A Major Lymphocytic Choriomeningitis Viral Epitope Restricted by H-2^b Haplotype Resides in the Glycoprotein between aa Residues 272-293.   A peptide encompassing LCMV ARM GP aa 272-293 LSDSSGVENPGGYCLTKWMILA was synthesized, and its activity was analyzed. When 200 µg/ml of peptide was used to coat H-2^b cells, these targets were lysed in an LCMV peptide-specific and H-2-restricted manner by LCMV CTL clones (specific ^{51}Cr released by clone 232: 62%, by clone 228: 40%) that recognize the major GP CTL epitope but not by the CTL clone (2-1: 7%) that recog-

TABLE I

Characteristics of CTL Clones Used

H-2^b syngeneic target cells inoculated with:	CTL clones reactive/total	Percent ^{51}Cr release with H-2^b syngeneic effector CTL clones:					
Vector:LCMV expression		232	228	31	RG-9	39	2-1
LCMV arm	18/18	47	60	55	66	37	41
Vaccinia/GP1 aa 1-262 + GP2 aa 263-363	17/18	49	52	43	55	22	0
Vaccinia/GP1 aa 1-262 + GP2 aa 263-293	17/18	52	55	41	59	28	8
Vaccinia/GP1 aa 1-262 + GP2 aa 263-271	2/18	0	0	0	15	9	9
Vaccinia/GP1 aa 1-218	2/18	0	0	0	12	ND	5
Vaccinia/NP aa 1-558	1/18	0	0	0	0	2	36
Vaccinia/NIL	0/18	0	0	0	0	10	0
Non-H-2 restricted (H-2^d targets)							
LCM virus	0/18						
Vaccinia/GP1 aa 1-262 + GP2 aa 263-293	0/18	Lysis by H-2^d					
Vaccinia/GP1 aa 1-262 + GP2 aa 263-272	0/18	Bulk splenic CTL: 67%					

In brief, the LCMV GP gene was serially truncated from the 3' end and attached to translational termination sequences. The restriction enzymes used were Bgl II, Pst I, Tth1 III, and Dra III which, respectively, generated COOH terminally truncated GP molecules comprising residues 1-363, 1-293, 1-271, and 1-218. These cDNA clones, and a full-length LCMV NP clone, were introduced into vaccinia virus by standard methods. In all cases, Northern blot analyses have confirmed the expression from the recombinant vaccinia of LCMV-specific RNA of appropriate size and polarity. All recombinants express abundant LCMV polypeptide as judged by fluorescent antibody detection. For details see references 23, 24.

nizes LCMV NP. The fact that CTL clones 232 and 228 lysed uninfected H-2^b targets coated with peptide aa 272-293 as efficiently as H-2^b targets infected with LCMV further confirmed that this peptide sequence contained the viral epitope being recognized by these clones. Results with CTL clones 31, RG-9, and 39 were similar (data not shown). The finding that primary splenic CTL gave lower values for target cells coated with peptide aa 272-293 when compared with target cells infected with virus indicated that bulk CTL recognize other viral epitopes. For example, specific lysis by H-2^b splenic CTL on peptide-coated H-2^b target was 26% as compared with 68% for LCMV-infected target. In contrast, H-2^d splenic CTL failed to lyse H-2^b targets either coated with peptide or infected with LCMV. These observations were recorded in over five separate experiments. When H-2^b targets were coated with non-LCMV peptides, no lysis occurred.

To determine the minimal amount of aa 272-293 required to coat targets for CTL activity, log dilutions of peptide were made, incubated with uninfected ^{51}Cr-labeled H-2^b targets, and reacted with either CTL clones 232 or 228. Results of these experiments indicated that half-maximal lysis is achieved with 0.5 µg/ml of the peptide (concentration: 0.25 µM).

The Minimal aa Sequence of LCMV Viral Epitope Restricted by H-2^b Haplotype is GP 278-286 VENPGGYCL. With a major viral epitope identified in aa 272-293, a set of 12 peptides was synthesized (upper 12 peptides, Fig. 1) that contained single aa deletions from the NH₂ or COOH termini. 40 µg of each peptide was used to coat H-2^b-uninfected targets, which were then reacted with CTL clones 232 or 228, and the ^{51}Cr released was quantitated. Both clones gave equivalent results, indicating that removing aa 272-276 or 288-293 had no effect on the efficiency of CTL ac-

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                                                          % Specific
       277                        286                     ⁵¹Cr Release
        |                          |
        G  V  E  N  P  G  G  Y  C  L  T  K  W  M  I  L  A    61
        G  V  E  N  P  G  G  Y  C  L  T  K  W  M              57
        G  V  E  N  P  G  G  Y  C  L  T  K  W                 57
        G  V  E  N  P  G  G  Y  C  L  T  K                    50
        G  V  E  N  P  G  G  Y  C  L  T                       65
        G  V  E  N  P  G  G  Y  C  L                          35
        G  V  E  N  P  G  G  Y  C                             <1
        G  V  E  N  P  G  G  Y  C  L  T  K  W  M              54
     S  G  V  E  N  P  G  G  Y  C  L  T  K  W  M              54
  S  S  G  V  E  N  P  G  G  Y  C  L  T  K  W  M              51
D S  S  G  V  E  N  P  G  G  Y  C  L  T  K  W  M              57
L S D S  S  G  V  E  N  P  G  G  Y  C  L  T  K  W  M          66

        G  V  E  N  P  G  G  Y  C  L  T                       56
        G  V  E  N  P  G  G  Y  C  L                          27
        G  V  E  N  P  G  G  Y  C  L                          24
              V  E  N  P  G  G  Y  C  L                       42
                 E  N  P  G  G  Y  C  L                        3
                    N  P  G  G  Y  C  L                       <1
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FIGURE 1. Minimal LCMV GP sequence composing the CTL recognition epitope. For Exp. 1, the upper 11 peptides were synthesized as described by the tea bag method (26), and the last peptide was synthesized with an automated peptide synthesizer. For Exp. 2, the first two peptides were synthesized by automated peptide synthesizer while the last four were made by the tea bag method. Results were equivalent for E/T ratios of 5:1 and 2.5:1; hence, only those for clone 228 at 2.5:1 on H-2^b targets are shown. Lysis was specific for LCMV ARM GP aa 272-286 (CTL clone 228: 61%; clone 232: 55%; clone 31: 46%; clone RG-9: 64%; clone 39: 35%) or larger peptides constructed by addition of native aa to NH$_2$ or COOH termini. Lysis was restricted to H-2^b targets and not H-2^d (lysis <8%) targets.

tivity. Thus, peptide aa 277-287, GVENPGGYCLT-coated cells were lysed equivalently with corresponding targets coated with aa 272-293 (Fig. 1). When THR in position 287 was removed, lysis occurred but at roughly half the value (35% compared with 65% specific ^{51}Cr release). When LEU in position 286 was removed, lysis was abolished. Repeated studies confirmed these observations. In other experiments, six additional peptides were generated (lower six peptides, Fig. 1) and their activity on targets with CTL clones showed that deletion of GLY from position 277 did not abrogate lysis (percent specific lysis by CTL clones 228, 232 GVENPG-GYCLT: 58%, VENPGGYCLT: 61%), whereas removal of VAL from aa position 278 (ENPGGYCLT: 3%) did. Thus the minimal epitope mapped comprises nine aa, 278-286 VENPGGYCL (Fig. 1).

Site-specific Substitution in aa Position 278 Defines Three Sets of CTL Clones. The next series of experiments used five CTL clones to focus on the fine structure of the viral epitope for CTL recognition. Since the previous studies indicated that VAL in position 278 was critical for CTL recognition of the viral epitope, a series of amino acid replacements at aa 278 were made to dissect peptide-directed CTL activity. As seen in Table II, conservative aa substitutions in position 278 VAL to ALA, LEU, or ILE did not alter CTL activity of any of the five clones. Substitution to GLY maintained the activity but at a lesser degree. From these aa replacements it is clear that these CTL clones prefer hydrophobic residues. Similarly, substitution of VAL to ASN resulted in only minor alterations in CTL lysis while change of VAL to LYS diminished killing by CTL clone 228 to a much greater extent than the other four CTL clones. Substitution of VAL to ASP or GLU diminished CTL activity in CTL clones 232, 31, RG-9, and 39 while totally abrogating response of CTL clone 228. Interest-

TABLE II

Finer Specificities of Five-CTL Clones that Recognize GP aa VENPGGYCL

Substituted amino acid	% Specific lysis by CTL clones				
278	232	31	RG9	228	39
| . . . GVENPGGYCL . . .	100	100	100	100	100
L	100	97	80	87	99
I	100	75	84	100	61
A	100	78	67	100	100
G	38	ND	ND	38	ND
S	62	100	95	2	53
T	33	86	91	2	75
F	70	79	67	1	<1
Y	96	34	23	2	<1
D	16	21	10	2	13
E	43	20	21	1	10
K	91	77	85	13	100
N	97	86	100	100	100

Fine specificities for CTL recognition. A series of peptides 272–293 were synthesized with single amino acid substitution in position 278 where VAL (V) was substituted for LEU (L), ILE (I), ALA (A), GLY (G), SER (S), THR (T), PHE (F), TYR (Y), ASP (D), GLU (E), LYS (K), or ASN (N) as described (26). Individual peptides at final concentration of 200 µg/ml were added to 2 × 10^4 ^{51}Cr-labeled H-2^b or H-2^d uninfected target cells. Cells were then incubated with either CTL clones 232, 228, 31, RG-9, or 39 (E/T ratio 2.5:1) for 4–5 h, and specific ^{51}Cr release was quantitated. Lysis was restricted to H-2^b targets (not H-2^d) with specific release of 40–60% for CTL clones reacted with the native aa sequence (i.e., VAL in position 278).

ingly, the CTL activity of clone 39 could be segregated from clones 232, 31, and RG-9 by amino acid substitutions that added aromatic amino acids in position 278. Thus, when aromatic amino acids were added as indicated by substitution of TYR or PHE for VAL, CTL clones 232, 31, and RG-9 retained their lytic activity, whereas, in contrast, CTL clone 39 totally lost activity. Similarly, when TYR or PHE replaced VAL or if a change in polarity occurred (substitution of SER or THR for VAL), CTL clone 228 lost total activity.

We then assayed the ability of CTL clones to lyse H-2^b-uninfected target cells coated with concentrations of LCMV GP peptide at 12.5, 50, or 200 µg/ml. As seen in Fig. 2, significant lysis by CTL clones 228 and 232 occurred over a wide dose range (from 12.5 to 200 µg/ml) with the majority of peptide-coated targets (Fig. 2). An exception was the lysis by CTL clone 232 of targets coated with LCMV GP peptide containing either SER or THR in residue 278. In this instance lysis occurred at 200 but not 50 or 12.5 µg/ml concentrations.

The Viral Epitope Recognized by H-2^b is Class I MHC Restricted to D^b, not K^b; and H-2D^b α1 aa 37–52 Peptides Block CTL Lysis. A final series of experiments dissected the H-2^b haplotype's contribution to CTL lysis using CTL clones 232 and 228. Results with target cells recombinant at the class I and class II MHC loci indicated that lysis was restricted to D^b, and not K^b or I^b. Hence, recombinant target B10.4R K^{k}I^{k}D^b cells were lysed as efficiently as the nonrecombinant K^{b}I^{b}D^b targets, whereas B10.3R and B10.5R both containing K^b and I^b are not lysed (Table III). D^b haplo-
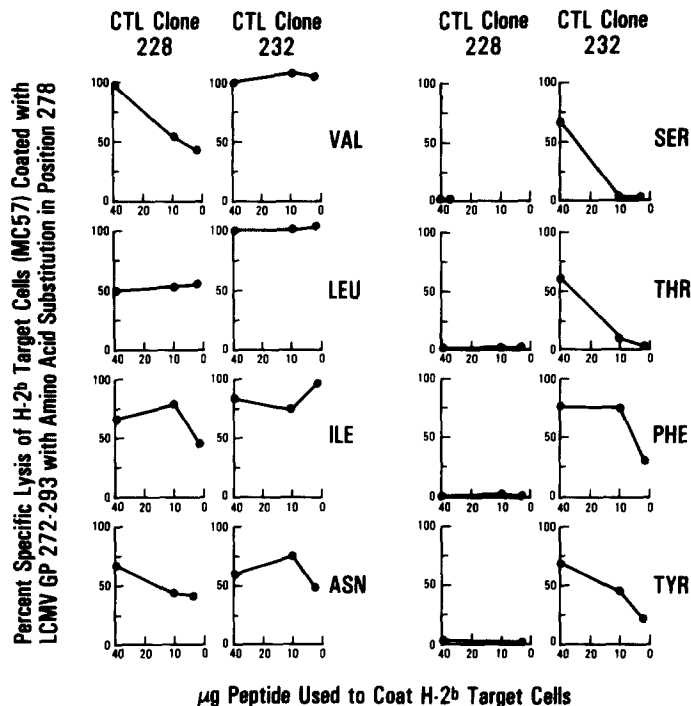
FIGURE 2.   Dose-response analysis of the peptides used to map fine specificities of CTL recognition. A series of peptides 272-293 was synthesized with single aa substitution in position 278 where VAL was replaced with LEU, ILE, ASN, SER, THR, PHE, or TYR as described (26). Individual peptides at final concentrations of 200, 50, or 12.5 µg/ml were added to 2 × 10⁴ ⁵¹Cr-labeled H-2ᵇ-uninfected target cells or 200 µg/ml added to 2 × 10⁴ ⁵¹Cr-labeled H-2ᵈ uninfected target cells. Cells were then incubated with either CTL clone 232 or 228 E/T ratio 2.5:1) for 4-5 h, and specific ⁵¹Cr release was quantitated. Lysis was restricted to H-2ᵇ targets (not H-2ᵈ) with specific release of 45% and 49% for CTL clones 228 and 232 reacted with native aa sequence (i.e., VAL in position 278).

types also contain Lᵇ class I molecules that cannot be separated by these experiments.

Comparison of LCMV GP epitope aa 272-293 with Dᵇ and Kᵇ sequences (Lᵇ has not yet been sequenced) stored in the Dayhoff data bank indicated regions of homology with Dᵇ and Kᵇ α1 amino acid sequence 37-52 (Fig. 4).

Corresponding Dᵇ and Kᵇ peptides were synthesized, purified by HPLC (>90% pure), and studied to see whether they interfered with lysis of H-2ᵇ-uninfected targets coated with LCMV GP aa 272-293 or infected with LCMV ARM. As seen in Fig. 4, H-2Dᵇ α1 aa 37-52 blocked lysis in a dose-response manner, but the corresponding Kᵇ peptide that differs from Dᵇ by substitution of ARG for PRO in res-

LCMV GP   274   DSSGVENPGGYCLTK WM

Dᵇ α1   37   D̂ŜD AÊN̂P̂ R̂ŶEPRAP̂ŴM̂

Kᵇ α1   37   D̂ŜD AÊN̂P̂ R̂ŶEPRAR̂ŴM̂

FIGURE 3.   Comparison of LCMV GP epitope aa 272-293 with Dᵇ and Kᵇ sequences stored in the Dayhoff data bank.

CTL Killing Is Restricted to Class I MHC D^b

Effector CTL	Ratio E/T	MC57 K^bI^bD^b		B10.3R K^bI^bD^d		B10.4R K^kI^kD^b		B10.5R K^bI^bD^d	
		LCMV	UNINF	LCMV	UNINF	LCMV	UNINF	LCMV	UNINF
Clone 232	5:1	83	5	5	8	90	8	7	5
	2.5:1	84	1	2	1	56	1	4	4
Clone 228	5:1	63	—	1	1	72	3	3	2

CTL clones at effector to target (E/T) ratios of 5:1 or 2.5:1 were used in a 5-h ⁵¹Cr-release assay as described (10, 11). Numbers represent mean values for triplicate samples. Variance was <10%.

idue 50 did not. These results were confirmed in several independent experiments. The D^b peptides also blocked killing of H-2^b splenic CTL, although some of these are K^b restricted (27).

Discussion

These studies provide a detailed fine structural map of a major LCMV ARM glycoprotein epitope recognized by H-2^b-restricted CTL. The epitope comprises the
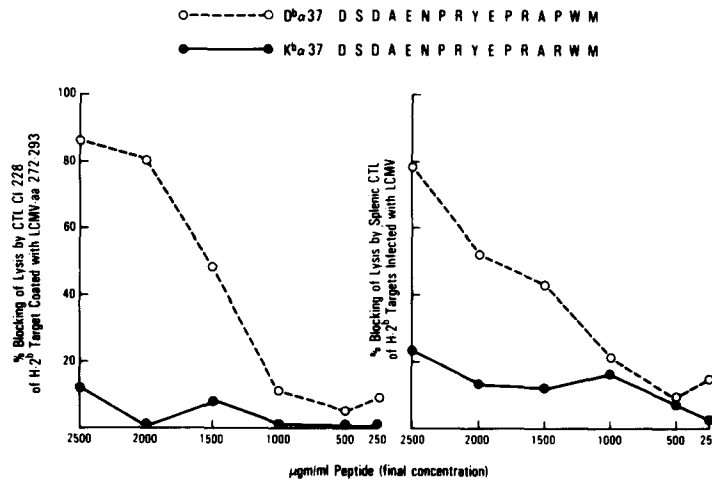

FIGURE 4. D^b peptide containing residues 37-52 from α1 domain blocks lysis of H-2^b targets coated with LCMV ARM GP aa 272-293 by CTL clone 228 or of LCMV-infected cells by bulk splenic CTL. CTL clones (5 × 10⁴ cell; E/T ratio 2.5:1) or bulk splenocytes 10⁶ cells; E/T 50:1) were incubated with varying final concentrations of D^b or corresponding K^b peptide (2,500; 2,000; 1,500; 1,000; 500, 250 µg/ml). After a 30 min incubation at 37°C the mixture was added to ⁵¹Cr-labeled uninfected MC57 cells coated with LCMV ARM peptide at 200 µg/ml and specific lysis was calculated. Similar results were observed with CTL clone 232.

linear amino acid sequence VENPGGYCL, aa 278–286 of LCMV ARM GP, and its recognition is restricted by the class I H-2 D^b gene product. The structure of this peptide bound to a target or within the native protein expressed on virus-infected cells is unknown. Nevertheless, the prevalence of GLY residues coupled with a PRO-GLY is most consistent with a β turn and unlikely to be an α-helical structure (28). Hence, the current predictive schemes involving an amphipathic α helix (29, 30) as used for T helper lymphocyte epitopes are not consistent with the experimental data obtained here and thus may be of limited value for CTL predictions. An alternative scheme (31) required hydrophobic residues flanked by GLY, charged or polar moieties. This motif is not found in the nine-aa epitope found for LCMV GP although it was helpful for predicting a CTL-influenza virus matrix protein (16). Clearly more epitopes need to be defined and analyzed before workable rules can be developed for a universally helpful prediction scheme. The approaches reported here coupled with studies in other viral systems (13–16, 32–34) should supply the background data required. Interestingly, the viral epitope we mapped according to normalized frequencies of occurrence of aa residues in secondary structure (28) suggested a likely β turn, as did aa in position 281–283 that contained PRO-GLY-GLY, which corresponds to similar sequences observed by the nuclear magnetic resonance studies of Dyson et al. (35, 36) for the formation of reverse turn of peptide fragments of proteins in water solution.

A major function of protein(s) encoded by the MHC is to bind selectively to a part of a viral (foreign) antigen, thereby making it recognizable to CTL. Analysis of the three-dimensional structure of the human HLA A2 molecule (37, 38) suggests that an MHC molecule has a single binding site for foreign antigen. Within the MHC structure is a groove 25 Å long, 10 Å wide and 11 Å deep capable of accommodating a peptide of 8 to 15 or 16 aa in length. The nine-aa epitope described here would fit into that size cavity.

To address the paradox of how a few MHC molecules might be able to handle a large repertoire of viral antigens, several investigators have proposed the existence of meaningful sequence homology between the foreign viral protein and MHC binding site (39–41). Accordingly, we found homology between the MHC H-2D^b restriction molecule in the α1 domain residues 37–52 and the H-2K^b nonrestriction molecule in the same location with LCMV GP 274–291. After adding spacers in the MHC molecules between residues 39–40 and 43–44, an alignment occurred where the minimal LCMV CTL epitope VENPGGYCL contained homologous residues -XENP-XY. The D^b peptide 37–52 differed from the K^b peptide by one amino acid in position 50 PRO → ARG and blocked CTL killing, while the K^b peptide did not. However, these results did not explain the observations that H-2^b splenic CTL (some of which are K^b and perhaps L^b restricted) are effectively blocked and that the single aa substitution between K^b and D^b maps a few aa upstream from the viral epitope (Fig. 3). Further, the H-2^b peptide maps close to but outside the cavity believed to be the HLA-A2 antigen binding site (37, 38). Conceivably, the activity of the PRO in position 50 of H-2D^b may provide a conformational change that empowers this peptide to inhibit competitively viral peptide binding to H-2^b MHC molecules expressed on the cell surface. The binding affinities of these peptides are not known nor is their mode of action. Presumably the affinity of the H-2D^b pep-

tide is higher than that of H-2K^b, thereby enabling the former to block recognition of the critical MHC molecule by CTL.

Where and how cells bind peptides is uncertain. It is unlikely that T lymphocytes recognize foreign antigens as natural three-dimensional structures (12). The mechanism by which such proteins are degraded into short peptides and the cellular pathways they undertake to reach the cell's surface is unclear. Recently a vaccinia/LCMV cDNA expressing the short peptide aa 272–293 was constructed and used to infect H-2^b cells. These cells processed the truncated protein internally, after which it was recognized by CTL clones 228 and 232 as efficiently as the synthesized LCMV GP 272–293 aa peptide added exogenously (Whitton, J. L., M. B. A. Oldstone, unpublished data). Mapping the pathways of these exogenous and endogenous viral reagents within target cells and determining where they associate with MHC may illuminate this problem.

Finally, CTL have a fine specificity allowing discrimination at the single aa level. For example, the five CTL clones analyzed here were defined on the basis of similar recognition of the major LCMV glycoprotein epitope restricted by H-2^b. The size of the nine-aa viral epitope and the ability of a single aa deletion from the native structure either at the NH$_2$ or COOH termini suggest that a single interaction with D^b occurred. Yet, the specificities of the clones could be further distinguished by selective aa substitutions in position 278 that altered the recognition epitope. Thus, addition of polar side chains by substituting SER or THR for VAL segregated these clones into two functional groups. Further, addition of aromatic acids by substitution of PHE or TYR for VAL further segregated these permissive CTL clones functionally. Dose-response analysis of substituted peptides, especially hydrophobic ones, failed to alter the established recognition pattern even using peptides at 12.5 μg/ml concentration. Fine CTL specificity has been noted previously by Bastin et al. (15) for influenza virus nucleoprotein where a substitution of ASP to GLU in aa position 372 aborted class I CTL clone F5 lysis. However the 15-aa size of the peptide epitope was not sufficiently mapped to ascertain whether a single or multiple site of interaction with MHC occurred. Nevertheless, those data and ours strongly suggest that fine CTL specificity appears similar to that exhibited by mAbs and T helper cells (42) as regards recognition of single aa changes. The discrimination by these CTL clones will likely be reflected in analysis of their corresponding CTL receptors. We are currently pursuing such studies with CTL clones reported here.

Summary

We define a nine–amino acid (aa) sequence of VAL-GLU-ASN-PRO-GLY-GLY-TYR-CYS-LEU as a major epitope for immunologic recognition of lymphocytic choriomeningitis virus (LCMV) by H-2^b-restricted CTL. The epitope was characterized using molecular genetics to bracket broadly and chemistry to precisely identify aa residues 278–286 of the viral glycoprotein. The epitope's compostion is characteristic of a reverse (β turn) but not an amphipathic α helix. A series of peptides with a single aa substitution in position 278 of VAL with other nonpolar (hydrophobic) amino acids (LEU, ILE, ALA, or GLY) coat targets that are recognized and lysed by CTL clones recognizing this epitope. In contrast, substitution of VAL with either large aromatic amino acids (that add bulk: PHE, TYR) or polar side

chains (SER, THR) segregates CTL clones normally recognizing aa 278–286 into two groups, one that remains lytic (permissive) despite these changes and another that fails to lyse, indicating CTL can discriminate at a single aa. A change in charge at this position (VAL → ASP or GLU), in general, reduces CTL lysis while a change of VAL to LYS or ASN has minimal affect for four of the five CTL clones analyzed. CTL reactivity with the viral epitope is restricted by the D^b but not the K^b of the murine MHC haplotype. A 16-aa peptide of D^b that spans α1 residues 37–52 blocks CTL lysis, whereas the corresponding K^b peptide that differs from D^b in a single aa in position 50 does not.

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