VIROLOGY 234, 62–73 (1997)
ARTICLE NO. VY978627

The Signal Sequence of Lymphocytic Choriomeningitis Virus Contains an Immunodominant Cytotoxic T Cell Epitope That Is Restricted by both H-2D^b and H-2K^b Molecules

Denis Hudrisier,* Michael B. A. Oldstone,† and Jean Edouard Gairin*,[1]

*Institut de Pharmacologie et de Biologie Structurale, UPR 9062 CNRS 205 route de Narbonne, 31400 Toulouse, France; and †The Scripps Research Institute, Department of Neuropharmacology, Division of Virology, 10550 North Torrey Pines Road, La Jolla, California 92037

Received February 27, 1997; returned to author for revision April 17, 1997; accepted May 8, 1997

Infection of H-2^b mice with lymphocytic choriomeningitis virus (LCMV) generates three well-characterized H-2D^b-restricted immunodominant epitopes delineated in the NP, GP1, and GP2 proteins. Here we report that the H-2D^b-restricted GP1 epitope GP33-41/43 (KAVYNFATC/GI) located in the signal sequence of LCMV is also the immunodominant epitope recognized by CTL at the surface of the same infected cells in the context of H-2K^b restriction. The GP1 epitope bound to H-2D^b and H-2K^b molecules with comparable affinities. The respective binding processes involved different sets of peptide anchoring residues and required dramatically different conformations of the peptide backbone as well as rearrangement of residue side chains. The 10-mer peptide GP34-43 (AVYNFATCGI) was the optimal H-2K^b-binding sequence and the 8-mer peptide GP34-41 (AVYNFATC) the minimal sequence for optimal H-2K^b-restricted CTL recognition. Comparison of lytic activities of primary splenic anti-LCMV CTL from C57BL/6 (D^{b+}/K^{b+}), B10A.[5R] (D^{b-}/K^{b+}), and B10A.[2R] (D^{b+}/K^{b-}) mice against LCMV-infected or peptide-coated target cells expressing either one or the two MHC alleles revealed that the H-2K^b-restricted component of the anti-GP1 CTL response was mounted independently of but as efficiently as its H-2D^b counterpart. Analysis of the immune response against a GP1 variant that escapes CTL recognition showed that the GP1 epitope: (i) was likely the only immunodominant LCMV epitope in the context of H-2K^b, and (ii) could efficiently evade H-2D^b and H-2K^b-restricted CTL mediated lysis.

INTRODUCTION

Cytotoxic T lymphocytes (CTL) play a crucial role in eradication of virus by recognizing, at the surface of infected cells, viral antigenic peptides presented by major histocompatibility complex (MHC) class I molecules. The CTL response depends on the ability of the viral peptide first, to bind to the MHC molecule and second, to trigger T cell receptor (TCR) activation. Following viral infection, very few peptides (generally one or two) within a viral protein serve as CTL immunodominant epitopes in the context of a given MHC class I restriction (Cole *et al.,* 1994; Kast *et al.,* 1991; Mylin *et al.,* 1995; Oldstone *et al.,* 1995). Presentation of the peptide and its immunodominance depend on multiple molecular and cellular mechanisms (Barber and Parham, 1994). Among them, peptide–MHC interaction is a critical step that requires the presence of a specific MHC binding motif (Falk *et al.,* 1991) in the peptide sequence and is controled by positive or negative factors at peptides nonanchor positions (Hudrisier *et al.,* 1996; Ruppert *et al.,* 1993).

Usually, a viral antigen contains one single MHC binding motif and is thus presented to CTL in only one MHC context. However, it is possible to find peptide sequences that harbor more than one MHC binding motif. Indeed, the presence of multiple MHC binding motifs found within some viral sequences may allow a few of them to bind to multiple MHC molecules (Bergmann *et al.,* 1994; Colbert *et al.,* 1994; Delguercio *et al.,* 1995; Luescher *et al.,* 1996; Schumacher *et al.,* 1991; Sidney *et al.,* 1995), but led rarely to their recognition by CTL in the context of more than one MHC restriction (Missale *et al.,* 1993; Oldstone *et al.,* 1992; Sheil *et al.,* 1994). However, occurence of CTL recognition of antigenic peptides known to bind to different alleles of the same haplotype, has never been observed *in vivo* in the context of more than one allelic restriction (Bergmann *et al.,* 1994; Cole *et al.,* 1994; Matsumura *et al.,* 1992; Schumacher *et al.,* 1991).

In H-2^b mice, acute lymphocytic choriomeningitis virus (LCMV) infection leads to a CD8$^+$ CTL response (Fung-Leung *et al.,* 1994; Kyburz *et al.,* 1993; Tishon *et al.,* 1995) directed predominantly against three H-2D^b-restricted epitopes located in each of the three viral proteins: the nucleoprotein NP (Schulz *et al.,* 1989) and glycoproteins 1 and 2 (GP1 and GP2) (Klavinskis *et al.,* 1992; Oldstone *et al.,* 1988), of which the optimal sequences have been well delineated: NP396–404 (FQPQNGQFI) for NP, GP33–41/43 (KAVYNFATC/GI) for GP1, and GP276–286 (SGVENPGGYCL) for GP2 (Gairin *et al.,* 1995). In addition, it is known that an LCMV-specific, H-2K^b-restricted CTL response also exists (Byrne *et al.,* 1984; Klavinskis *et al.,* 1992), but its epitope had not been characterized.

[1] To whom correspondence and reprint requests should be addressed. Fax: +33 (0) 561-17-59-94. E-mail: gairin@ipbs.fr.

0042-6822/97 $25.00

Here we record that the H-2D^b-restricted epitope contained in the signal sequence of LCMV GP1 also harbored the H-2K^b binding motif. The GP1 peptide that bound to H-2D^b also efficiently bound to H-2K^b and comprised the dominant CTL response restricted by H-2K^b. To our knowledge, this is the first evidence that a single peptide can serve as an immunodominant epitope in the context of two MHC alleles copresented at the surface of the same infected cell to CTL. At the molecular level, the H-2K^b-restricted optimal sequence of the epitope was delineated using alanine-substitution scanning and N- and C-terminus truncation analysis. At the cellular level, the two allelic components of the anti-GP1 CTL response were dissected and showed that the viral antigen was presented to and recognized by CTL in the context of H-2D^b and H-2K^b equivalently. This GP1 epitope was found to be the only immunodominant LCMV epitope in the context of H-2K^b.

MATERIALS AND METHODS

Cell lines, murine, and viral strains

The murine mutant lymphoma cell line RMA-S (H-2^b) was used in stabilization experiments. The murine H-2^b cell line MC57 and the human cell line T2 transfected with H-2D^b (T2-D^b) or with H-2K^b (T2-K^b) were used as target cells in the CTL assays. Cells were grown in RPMI 1640 (RMA-S and MC57) or IMDM (T2-K^b, T2-D^b) containing 8% bovine serum, L-glutamine, and antibiotics. Geneticin (400 μg/ml) was added to IMDM to maintain selection of positively transfected T2-D^b and cells T2-K^b. C57BL/6 (H-2^{bb}) mice were from the Scripps Research Institute breeding colony. B10.A[2R] (D^b, K^k) and B10.A-[5R] (D^d, K^b) mice were obtained from Jackson Laboratories. LCMV Armstrong (ARM) wild-type strain clone 53b was used to infect mice or cells.

Peptides synthesis and labeling

Peptides were synthesized on an automated peptide synthetizer (Applied Biosystems 430A) by the solid-phase method using either t-BOC or FMOC chemistry, purified by HPLC on a RP300-C8 reversed-phase column (Brownlee Lab), and their identity confirmed by fast atom bombardment or electro-spray mass spectrum analysis as previously described (Gairin *et al.,* 1995).

MHC binding study

Peptide binding to MHC was studied in stabilization experiments as previously described (Hudrisier *et al.,* 1995). Briefly, RMA-S cells were grown at 25° for 24 hr before the assay to induce stable H-2K^b or H-2D^b molecules at the cell surface. Cells (5×10^5 cells/well) were incubated at 37° in microtiter plates and the stability of MHC molecules at this temperature was studied as a function of peptide concentration (10^{-9} to 10^{-4} *M*) after a 4-hr incubation period in presence of protease inhibitors (1 m*M* Pefabloc, 0.1 *M* bestatine, 1 m*M* EDTA, 0.3 m*M* aprotinin). Cells were then incubated on ice for 1 hr with 0.1 ml of hybridoma culture supernatant of mouse monoclonal antibodies Y3 specific for α1 and α2 domains of H-2K^b (Hammerling *et al.,* 1982) or 28-14-8S specific for the α3 domain of H-2D^b (Ozato *et al.,* 1980), respectively. Negative control was done with medium alone or irrelevant antibody. After one wash with ice-cold BSA-PBS, cells were incubated for 1 hr with a fluorescent secondary antibody (fluorescein isothiocyanate-conjugated goat anti-mouse IgG, Sigma). After two washes, cells were fixed in 1% paraformaldehyde in BSA-PBS and analyzed by fluorescence-activated cell sorter (FacScan, Becton–Dickinson). Fifty percent stabilizing concentration (SC_{50}) corresponds to the peptide concentration for which half of the maximum up-regulation is reached.

In vitro cytotoxicity assays

MC57, T2-D^b, and T2-K^b target cells were incubated for 1 hr at 37° with ^{51}Cr, washed three times with culture medium, and placed in flat-bottom 96-well plates. LCMV-infected cells or uninfected target cells pulsed with increasing concentrations (as indicated in figure legends) of synthetic peptides were subjected to lysis by LCMV-specific H-2^b-restricted primary splenocytes. Bulk splenocytes were used at an E:T ratio of 50 to 1. MC57 cells infected 48 hr before the assay with the LCMV Armstrong strain (multiplicity of infection = 2) were used as positive controls. Uninfected cells in the absence of peptides (50 μl of culture medium) were used as negative controls. Target and effector cells were incubated at 37° in a final volume of 200 μl. After a 5-hr incubation period, fractions (100 μl per well) were removed and counted for ^{51}Cr activity. The percentage of specific lysis was calculated as $100 \times$ [(cpm, experimental release $-$ cpm, spontaneous release)/(cpm, total release $-$ cpm, spontaneous release)]. Total and spontaneous releases were determined by incubating the labeled cells with 1% Nonidet 40 and culture medium, respectively. In all experiments, samples were run in triplicate, and means of the values are given.

Molecular modeling

Molecular modeling of interactions between the LCMV GP1 peptide and H-2K^b or H-2D^b was performed using Insight II (Biosym Technologies, CA) as described (Hudrisier *et al.,* 1996). Starting coordinates were taken from the crystal structure of H-2D^b in complex with Influenza NP366–374 (ASNENMETM) (Young *et al.,* 1994) and from H-2K^b in complex with Vesicular Somatitis Virus (VSV) NP52–59 (RGYVYQGL) (Zhang *et al.,* 1992). The sequences of the LCMV GP1 peptide selected for modeling its interaction with H-2D^b or H-2K^b were GP33–41 (KAVYNFATC) and GP34–41 (AVYNFATC) and their structures were built by coordinates assignment using HOMOLOGY (Biosym) from model peptides ASNENMETM and RGYVYQGL, respectively. Structures were manually refined

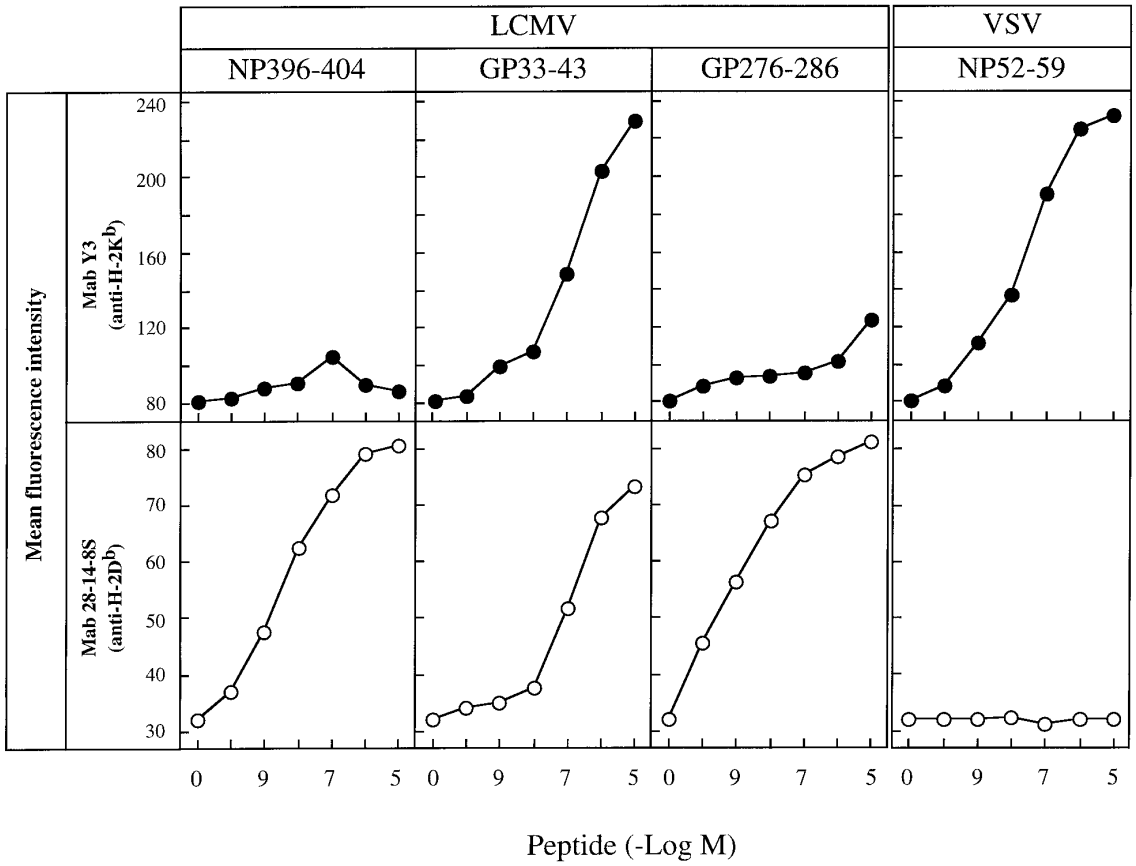

FIG. 1. Binding of the H-2D^b-restricted LCMV and H-2K^b-restricted VSV viral epitopes to H-2K^b and H-2D^b. RMA-S cells were incubated with increasing concentrations (10^{-10} to 10^{-5} *M*) of LCMV NP396–404 (FQPQNGQFI), GP33–43 (KAVYNFATCGI), GP276–286 (SGVENPGGYCL), and VSV NP52–59 (RGYVYQGL) at 37° for a 4-hr incubation period. Peptide-mediated up-regulation of H-2K^b (upper panel) and H-2D^b (lower panel) was assessed by flow cytometry using Y3 (anti-H-2K^b) and 28-14-8S (anti-H-2D^b) murine monoclonal primary antibodies followed by anti-mouse FITC-labeled secondary antibody. Results, expressed as mean fluorescence intensity, are representative among three independent experiments.

before submitted to 100 cycles of energy minimization using DISCOVER (Biosym).

RESULTS

The H-2D^b-restricted LCMV GP1 epitope binds to H-2K^b with high affinity

The ability of the three H-2D^b-restricted LCMV epitopes to bind to either H-2D^b or H-2K^b was determined in stabilization experiments on the murine mutant cell line RMA-S. The absence of cross-reactivity between the two monoclonal antibodies Y3 and 28-14-8S allowed an unambiguous determination of specific peptide-mediated upregulation of H-2K^b and H-2D^b expression, respectively. The H-2K^b-restricted epitope VSV NP52-59 (Zhang *et al.,* 1992) was used as control peptide. As shown in Fig. 1, the three H-2D^b-restricted LCMV epitopes were able to induce a dose-dependent stabilization of H-2D^b molecules at the surface of RMA-S cells, as expected from previous studies (Gairin *et al.,* 1995; Hudrisier *et al.,* 1996) while the H-2K^b-restricted epitope VSV NP52–59 used as negative control remained uneffective. In contrast, stabilization of H-2K^b was observed only in the presence of the LCMV GP1 epitope GP33–43 or of the positive control peptide VSV NP52–59. The two other LCMV epitopes NP396–404 and GP276–286 were totally unable to stabilize H-2K^b even at the highest concentration tested. Stabilization of H-2K^b and H-2D^b by the GP1 epitope occurred in the same range of peptide concentrations, reflecting a comparable affinity of the viral peptide for the two alleles. Similar results were obtained when the 9-mer sequence GP33–41 of the epitope was used (not shown).

Binding of the LCMV GP1 epitope to H-2K^b and H-2D^b involves different sets of residues

To study the role of each residue of the GP1 epitope in H-2K^b and H-2D^b binding, we analyzed the effect of monoalanine substitution on the MHC binding properties of the undecameric sequence GP33–43 (KAVYNFATCGI) of the antigen (Gly was used for substitution of Ala^{34} and Ala^{39}). Figure 2 shows that binding to H-2K^b was negatively affected by alanine substitution at four positions. The drop in affinity observed after substitution of Tyr^{36}, Phe^{38}, or C-terminal Ile^{43} was in accord with the expected role of these positions as main (Phe^{38} and Ile^{43}) and auxilliary (Tyr^{36}) anchors to H-2K^b (Falk *et al.,* 1991;

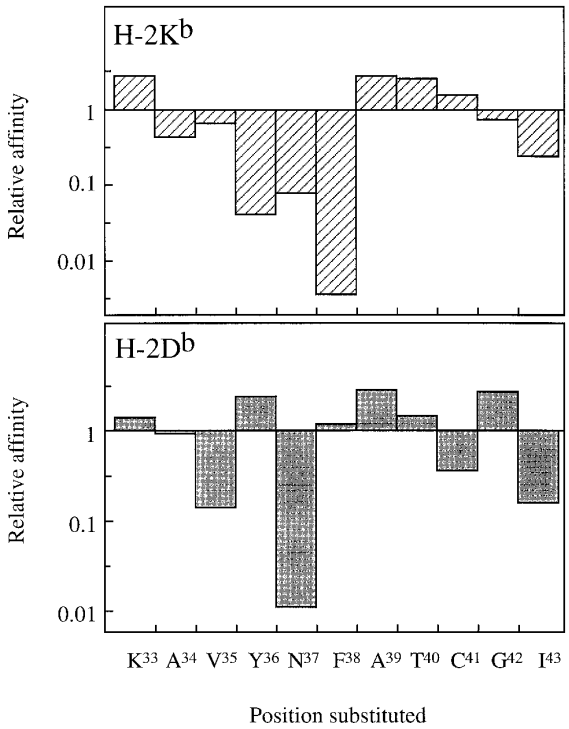

FIG. 2. Effect of mono alanine substitution on the H-2K^b and H-2D^b binding properties of the LCMV GP33-43 epitope. Alanine-substituted (or Gly-substituted for Ala^{34} and Ala^{39}) analogues were tested in stabilization assays as described under Materials and Methods and in the legend of Fig. 1. Results are expressed as relative affinity for H-2K^b (upper panel) or H-2D^b (lower panel) of an analogue vs wild-type peptide by the ratio $SC_{50wt}/SC_{50analogue}$. SC_{50wt} values for H-2K^b and H-2D^b were 214 $\pm$ 30 n*M* and 840 $\pm$ 168 n*M*, respectively (see Table 1). SC_{50} is the peptide concentration giving 50% maximal up-regulation. They represent one representative experiment among three.

Jameson and Bevan, 1992; Saito *et al.,* 1993; Shibata *et al.,* 1992). The important effect noted after substitution of Asn^{37} (located between the two anchors Tyr and Phe) occurred presumably via an indirect conformational effect. No dramatical change was observed after substitution at other positions, H-2K^b affinity being slightly modified (affinity ratio <3), either higher (Lys^{33}, Ala^{39}, Thr^{40}, Cys^{41}) or lower (Ala^{34}, Val^{35}, Gly^{42}). Concerning H-2D^b binding, four residues were clearly affected by alanine substitution, demonstrating their role as main (Asn^{37}), auxilliary (Val^{35}), or potent C-terminal (Cys^{41}, Ile^{43}) H-2D^b anchors, as expected from previous studies (Gairin *et al.,* 1995). All the other substitutions led to peptides with comparable (Lys^{33}, Ala^{34}, Phe^{38}, Thr^{40}) or slightly enhanced (Tyr^{36}, Ala^{39}, Gly^{42}) H-2D^b binding affinity.

The H-2D^b-restricted LCMV GP1 peptide is also an immunodominant H-2K^b-restricted epitope

Having established that the H-2D^b-restricted LCMV GP1 epitope also bound to H-2K^b, we then determined its ability to generate an anti-LCMV, H-2K^b-restricted CTL response. C57BL/6 (D^{b+}/K^{b+}), B10A.[5R] (D^{b-}/K^{b+}), and B10A.[2R] (D^{b+}/K^{b-}) mice were infected with LCMV and their splenocytes taken 1 week later and tested in a classical CTL assay. In the first set of experiments shown in Fig. 3, we confirmed that, following acute LCMV infection, an anti-LCMV CTL response was generated in the context of H-2K^b. LCMV-infected MC57 (D^{b+}/K^{b+}) target cells were efficiently lysed by bulk splenocytes from C57BL/6 (D^{b+}/K^{b+})-, B10A.[5R] (D^{b-}/K^{b+})-, and B10A.[2R] (D^{b+}/K^{b-})-infected mice showing that the H-2^b primary CTL response mounted against LCMV consisted of two components: the known H-2D^b-restricted response (seen in B10A.[2R]) and an H-2K^b one (observed in B10A.[5R]). In a second set of experiments, these splenocytes were then tested against target cells expressing either only one (T2-K^b, T2-D^b) or both (MC57) of the two alleles and coated with LCMV GP33-43 or NP396-404 used as a control. Results are presented in Fig. 4. Data shown in Figs. 4a, 4c, 4g, and 4i, confirmed the known anti-LCMV, H-2D^b-restricted CTL response against these epitopes since peptide-coated MC57 and T2-D^b target cells were lysed by CTLs from LCMV-infected C57BL/6 or B10A.[2R] mice. Data shown in Figs. 4b, 4d, and 4e demonstrated that the CTL response mounted *in vivo* in the context of H-2K^b was indeed directed against the GP1 peptide, giving similar patterns in the presence (Fig. 4d) or absence (Fig. 4e) of the H-2D^b-restricted response at the peptide concentrations tested. As shown in Figs. 4c and 4i, the H-2D^b-restricted response against GP1 was diminished in absence of H-2K^b, reflecting a possible modulation of the hierachy between the epitopes (Lewicki *et al.,* 1995a; Oldstone *et al.,* 1995). Finally, as shown unambiguously in Figs. 4f and 4h, peptide-coated target cells expressing only one of the two alleles (H-2K^b in Fig. 4f; H-2D^b in Fig. 4h) were not at all recognized nor killed by CTLs restricted by the other allele (H-2D^b in Fig. 4f; H-2K^b in Fig. 4h). In control experiments, MC57, T2-D^b, or T2-K^b target cells either uncoated or coated with irrelevant peptide

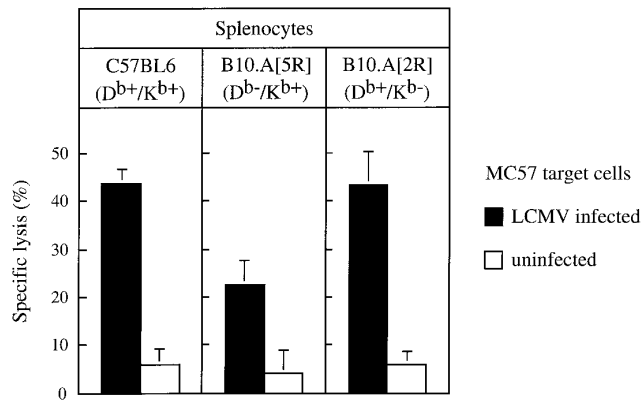

FIG. 3. H-2K^b and H-2D^b-restricted CTL response against LCMV. C57BL/6 (D^{b+}/K^{b+}), B10A.[5R] (D^{b-}/K^{b+}) or B10A.[2R] (D^{b+}/K^{b-}) mice were infected with LCMV Arm. Seven days latter, their splenocytes were tested against ^{51}Cr-labeled MC57 (D^{b+}/K^{b+}) cells infected (black bars) or not (white bars) with LCMV Arm in a classical 5-h CTL assay (E:T ratio = 50:1). Results, expressed as a percentage (%) of specific lysis, are the mean $\pm$ SD of two independent experiments.

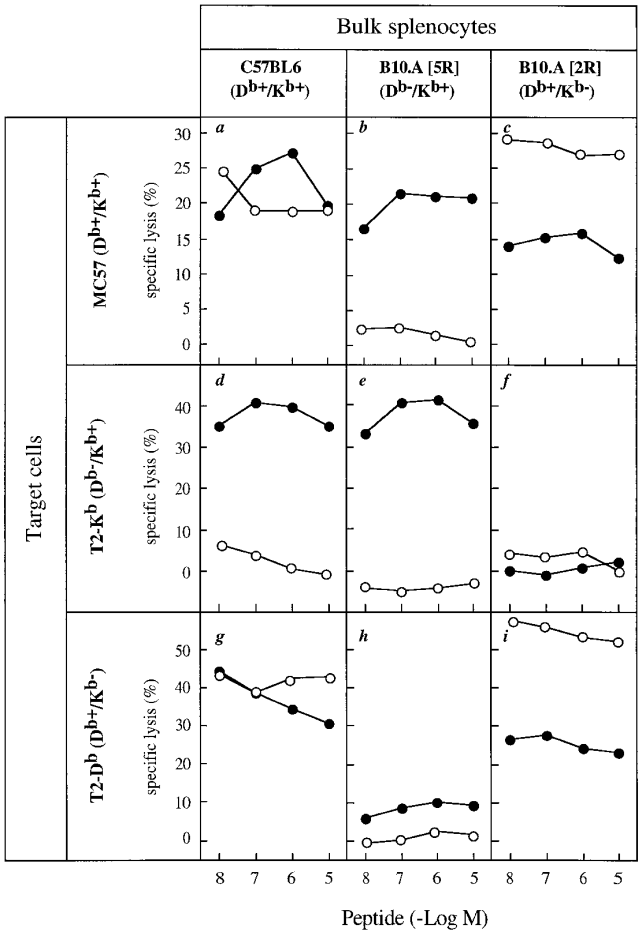

FIG. 4. Characterization and dissection of the H-2K^b- and H-2D^b-restricted anti-LCMV CTL response. Bulk splenocytes from C57BL/6 (D^{b+}/K^{b+}), B10A.[5R] (D^{b-}/K^{b+}), or B10A.[2R] (D^{b+}/K^{b-}) mice infected with LCMV 7 days before the assay (1st, 2nd, and 3rd columns, respectively) were tested against ^{51}Cr-labeled MC57 (D^{b+}/K^{b+}), T-2K^b (D^{b-}/K^{b+}) or T-2D^b (D^{b+}/K^{b-}) target cells (1st, 2nd, and 3rd row, respectively) coated with increasing concentrations (10^{-8} to 10^{-5} *M*) of LCMV GP33–43 (closed symbols), or NP396–404 (open symbols) used as control in a classical 5-hr CTL assay (E:T ratio = 50:1). Results, expressed as percentage (%) of specific lysis are representative of two independent experiments.

or T2 target cells coated with LCMV peptides were not lysed (% specific lysis $< 5\%$) by splenocytes from LCMV-infected mice (data not shown).

The 10-mer GP34-43 peptide (AVYNFATCGI) is the optimal sequence for selective, high-affinity binding to H-2K^b

To determine the H-2K^b-restricted optimal sequence of the GP1 peptide in terms of affinity and selectivity (K^b/D^b), C- and N-terminus truncated analogues of GP33–43 were tested for their respectives H-2K^b and H-2D^b binding properties as described above (see stabilization experiments on RMA-S cells). As shown in Table 1 (step 1), GP33–43 displayed a 4-times higher affinity for H-2K^b than for H-2D^b. Deletion of the C-terminal residue Ile^{43} resulted in a dramatic drop in affinity for both alleles. An additional shortening led to the nonapeptide GP33–41 that regained in MHC affinity (compared to GP33–42) particularly for H-2D^b. Finally, a further deletion resulted in the octamer GP33–40 that bound only very weakly to either H-2K^b or H-2D^b. Based on these results, N-terminus analysis was then conducted on the GP33–43 sequence (step 2). Deletion of Lys^{33} slightly enhanced the H-2K^b binding properties with a simultaneous and complete drop in H-2D^b affinity, resulting in a peptide with good affinity for H-2K^b ($SC_{50} = 164 \pm 29$ n*M*) and a remarkable binding selectivity (H-2K^b vs H-2D^b > 600). A further shortening (deletion of Ala^{34}) resulted in a drop in H-2K^b affinity and consequently in selectivity. In a final step, the sequence GP34–43 was used for a fine delineation of the optimal H-2K^b-binding sequence of the GP1 peptide. As seen in Table 1 (step 3), none of the truncated analogues of GP34–43 exhibited a better H-2K^b-binding profile at both affinity and selectivity levels than GP34–43 did.

The 8-mer peptide GP34-41 (AVYNFATC) is the minimal sequence for optimal H-2K^b-restricted CTL recognition

C- and N-terminus truncated analogues were then tested for their ability to sensitize T2-K^b (D^{b-}/K^{b+}) target cells to lysis by bulk splenocytes from LCMV-infected B10A.[5R] (D^{b-}/K^{b+}) mice. Results are presented in Fig. 5. As shown in the left panel of Fig. 5A, deletion of the first two residues Ile^{43} and Gly^{42} did not result in a drop in the H-2K^b-restricted CTL sensitization properties, in contrast to the effect observed on the H-2K^b-binding properties (see Table 1, step 1). Further, in several independent experiments, the nonamer KAVYNFATC was slightly — but reproductibly — the most effective peptide, as illustrated in the figure. In contrast, deletion of Cys^{41} abolished almost completely CTL lysis. N-terminal analysis (shown in the right panel of Fig. 5A) revealed that deletion of Lys^{33} did not bring significant change in CTL sensitization, the shorter peptide GP34–43 being barely but consistently better than GP33–43 in repeated experiments. Deletion of Ala^{34} resulted in a peptide about 2 log less efficient in inducing CTL killing. As shown in Fig. 5B, fine delineation of the epitope did not lead to a clear-cut profile of the GP1 sequence optimal for CTL recognition. The 8-mer AVYNFATC was as efficient as the 10-mer AVYNFATCGI to sensitize target cells to CTL killing despite a 1 log lower MHC binding affinity (see Table 1, step 3). Finally, the shorter peptide, the 7-mer GP34-40 (AVYNFAT) still induced a good killing despite its very weak binding affinity for H-2K^b (see Table 1, step 3), in the same concentration range than that of the 9-mer GP34-42 (AVYNFATCG).

The central core sequence GP35–38 (VYNF) of the GP1 epitope adopts dramatically different conformations in the context of H-2K^b or H-2D^b complexes

To better understand at both the molecular and atomic levels how the same peptide can bind to two different

TABLE 1

H-2K^b and H-2D^b Binding Affinities of N- and C-Terminus Truncated Analogues of the LCMV GP1 Epitope

Peptide		Binding affinity H-2K^b (SC_{50}, n*M*)	Binding affinity H-2D^b (SC_{50}, n*M*)	Selectivity (H-2K^b vs H-2D^b)
Step 1: C-terminus analysis				
GP 33-43	KAVYNFATCGI	214 ± 30	840 ± 168	3.9
GP 33-42	KAVYNFATCG	77 750 ± 7200	19 200 ± 2 750	2.5
GP 33-41	KAVYNFATC	1 314 ± 179	459 ± 84	0.3
GP 33-40	KAVYNFAT	28 860 ± 1 450	64 000 ± 8 370	2.2
Step 2: N-terminus analysis				
GP 33-43	KAVYNFATCGI	214 ± 30	840 ± 168	3.9
GP 34-43	AVYNFATCGI	164 ± 29	>100 000	>600
GP 35-43	VYNFATCGI	4 240 ± 496	>100 000	>23
Step 3: fine delineation				
GP 34-43	AVYNFATCGI	164 ± 29	>100 000	>600
GP 34-42	AVYNFATCG	3 600 ± 248	>100 000	>28
GP 34-41	AVYNFATC	1 417 ± 122	>100 000	>71
GP 34-40	AVYNFAT	46 500 ± 2 180	>100 000	>2

Note. RMA-S cells were incubated with increasing concentrations (10^{-9} to 10^{-4} *M*) of LCMV GP 33-43 or its N- and C-terminus truncated analogues for 4 hr at 37°. Peptide-mediated up-regulation of H-2K^b and H-2D^b expression was assessed by flow cytometry as described in the legend of Fig. 1 and under Materials and Methods. Values are the mean ± SEM of at least three independent experiments.

alleles, the structure of GP1 in complex with H-2K^b or H-2D^b was analyzed by molecular modeling. Starting from crystallographic coordinates of VSV8-H-2K^b (Zhang *et al.,* 1992) and FLU-H-2D^b (Young *et al.,* 1994) complexes, we inserted the GP1 epitope in the respective binding grooves of its two restriction elements. The sequences AVYNFATC and KAVYNFATC were chosen for molecular modeling of GP1 binding to H-2K^b and H-2D^b, respectively, given their optimal recognition by CTLs and their identical length with peptides cocrystallized in the context of H-2K^b and H-2D^b (Young *et al.,* 1994; Zhang *et al.,* 1992). Both models are presented in Fig. 6. The modeled structure of GP1 in complex with H-2K^b (Fig. 6A) showed that side chains of residues Tyr^{36}, Phe^{38}, and Cys^{41} interacted strongly with the MHC and that those of Asn^{37}, Ala^{39}, Thr^{40}, and, at a lower degree, Ala^{34} were accessible to the TCR. In contrast, when GP1 was modeled in interaction with H-2D^b (Fig. 6B), a dramatic conformational change was observed at the level of the central core motif VYNF of the peptide sequence. In the H-2D^b con-

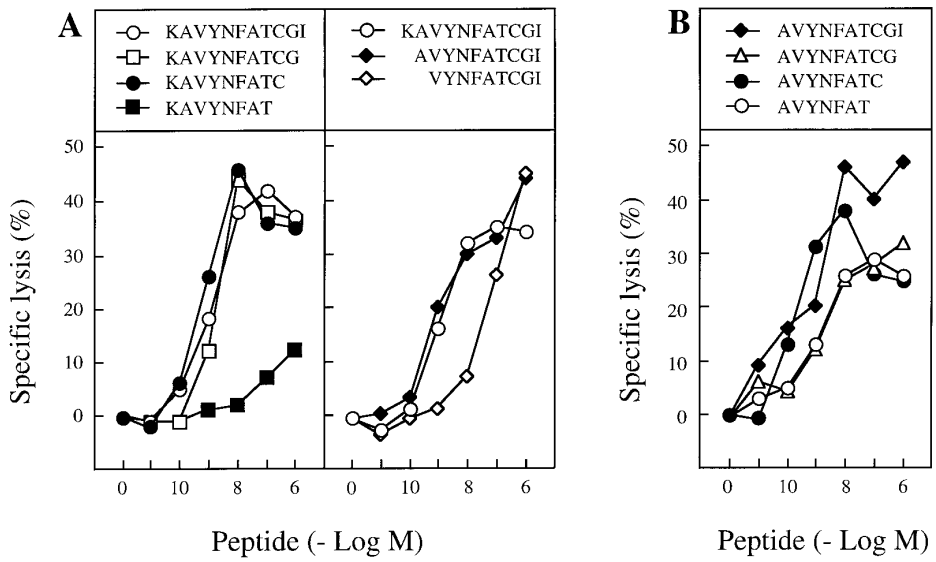

FIG. 5. Fine delineation of the H-2K^b-restricted LCMV GP1 epitope sequence. C- and N-terminus-truncated analogues of GP33–43 (A) and C-terminus-truncated analogues of GP34–43 (B) were tested for their ability to sensitize ^{51}Cr-labeled T-2K^b target cells to lysis by bulk splenocytes from LCMV-infected B10A.[5R] mice in a classical 5-hr CTL assay. Peptide concentrations varied from 10^{-11} to 10^{-6} *M.* E:T ratio was 50:1. Results, expressed as percentage of specific lysis are representative of two independent experiments.

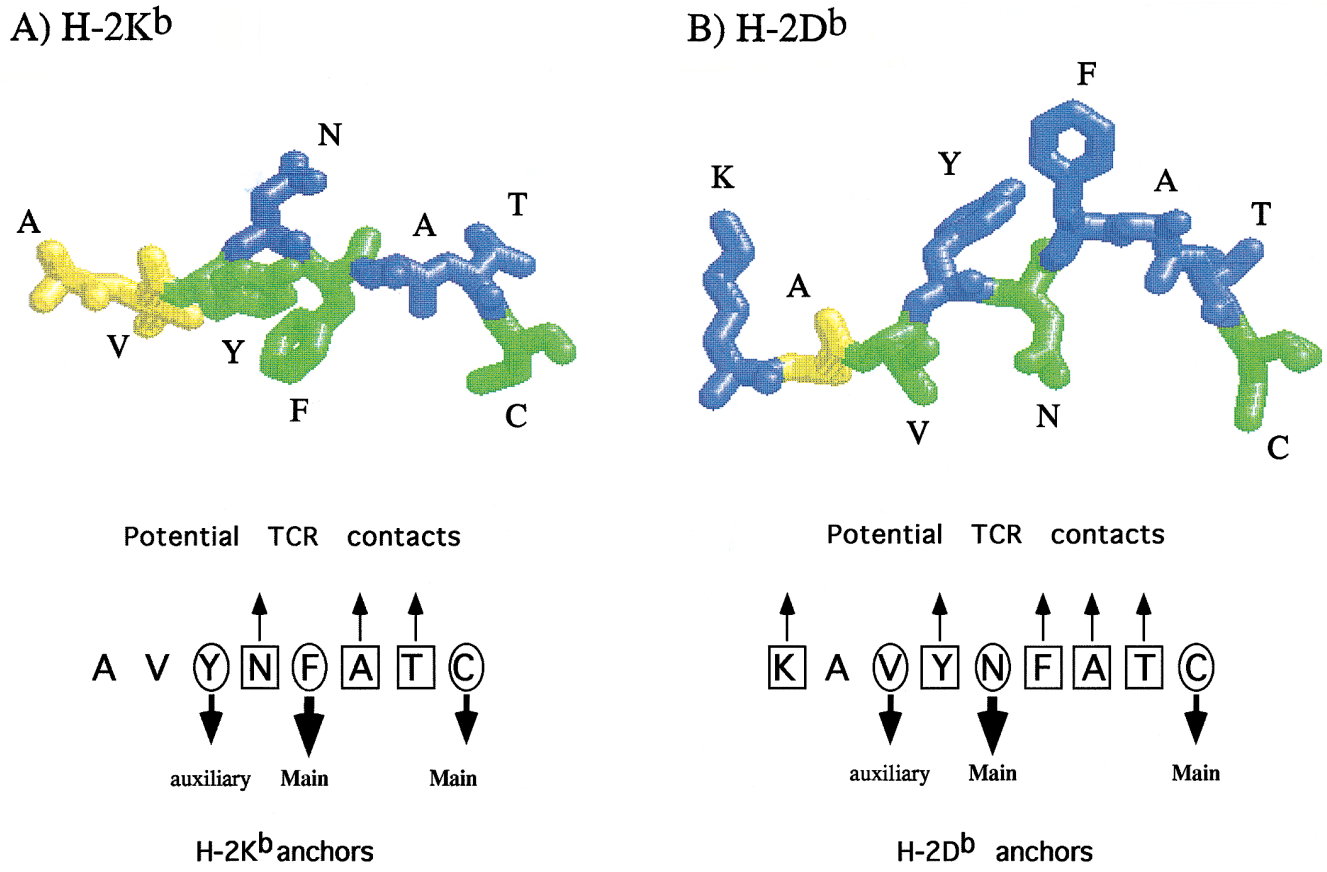

FIG. 6. Computerized molecular model of the LCMV GP1 epitope in complex with H-2K^b (A) or H-2D^b (B). Molecular modeling was performed using Insight II (Biosym Technologies, CA) starting from the coordinates of H-2K^b in complex with Vesicular Somatitis Virus (VSV) NP52–59 (RGYVYQGL) (Zhang *et al.,* 1992) and of H-2D^b in complex with Influenza NP366–374 (ASNENMETM) (Young *et al.,* 1992). The LCMV GP33–41 and GP34–41 structures were built by coordinates assignment from model peptides using HOMOLOGY (Biosym). A side view is shown. Schemes assigning the role of each residue of the epitope in either MHC binding or TCR recognition are represented in the bottom of the panels.

text, the side chains of Val^{35} and Asn^{37} were now oriented inside the MHC molecule together with Cys^{41} while those of Tyr^{36} and Phe^{38} were directed toward the TCR, together with Lys^{33}, Thr^{40}, and, to a lesser extent, Ala^{39}.

The LCMV GP1 variant (Phe^{38} → Leu^{38}) escapes CTL recognition by two independent mechanisms

The single mutation Phe^{38} → Leu^{38} in the LCMV GP1 protein sequence allows the virus to escape the H-2^b restricted, GP1-specific CTL response (Lewicki *et al.,* 1995; Oldstone *et al.,* 1995; and Fig. 7A). To dissect the molecular mechanisms by which escape may occur in the context of H-2D^b and H-2K^b, we analyzed the ability of the variant sequence to be presented by each of the two alleles. For that, the MHC binding properties of the mutated peptide [Leu^{38}]-GP33–41 (KAVYNLATC) were measured as described above and compared to those of the nonmutated sequence GP33–41 (KAVYNFATC). As shown in Fig. 7B, the mutation Phe^{38} → Leu^{38} did not affect presentation of the viral antigen by H-2D^b since both peptides bound to H-2D^b with comparable affinities. In this context, escape occurs via alteration of the crucial TCR contact residue that abolishes CTL recognition, an hypothesis in accord with previous studies (Lewicki *et al.,* 1995; Oldstone *et al.,* 1995) and illustrated in the molecular model (see above). In contrast, antigen presentation by H-2K^b was dramatically affected by the mutation since the mutated peptide showed a 3 log decrease in H-2K^b binding affinity compared to the nonmutated antigen. In this case, the mutation affects the main peptide anchor residue to the MHC (Falk *et al.,* 1991; Shibata *et al.,* 1992), in accord with the binding data and the molecular model (see sections above), and consequently abrogates its presentation to CTL.

DISCUSSION

In this study we have characterized the H-2K^b-restricted immunodominant epitope of LCMV. It shares the sequence of the H-2D^b-restricted GP1 epitope in the signal peptide of the viral GP protein, and the anti-GP1 T cell response that followed acute LCMV infection is mounted separately and efficiently in the two contexts. Our data show for the first time that a unique viral peptide can be efficiently presented *in vivo* at the surface of the

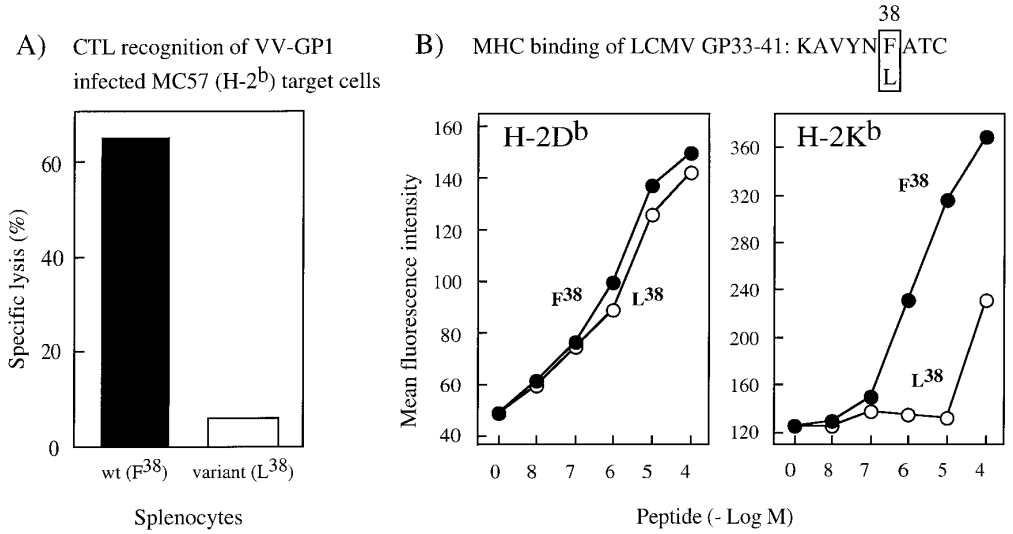

FIG. 7. The GP1 variant (Phe[38] → Leu[38]) escapes CTL recognition by two different mechanisms. In A, primary bulk splenocytes from C57BL/6 mice infected with LCMV wt (Phe[38], black bar) or GP variant (Leu[38], white bar) were tested for their ability to lyse H-2[b] MC57 target cells infected with vaccinia virus recombinants expressing LCMV GP1 (Oldstone *et al.,* 1995) in a classical 5-hr CTL assay (E:T ratio = 50:1). In B, the H-2D[b] or H-2K[b] binding properties of the LCMV wt GP1 peptide sequence GP33–41 (KAVYNFATC, closed symbols) and its CTL escape variant mutated form [Leu[38]]-GP33–41 (KAVYNLATC, open symbols) were evaluated in stabilization assays as described under Materials and Methods and in the legend of Fig. 1.

same infected cell by two different MHC alleles and can induce an immunodominant CTL response in both instances. Despite reports of the presence of multiple MHC binding motifs in a single sequence, only a few antigenic determinants are presented *in vivo* by MHC molecules of different haplotypes (Missale *et al.,* 1993; Oldstone *et al.,* 1992; Shirai *et al.,* 1992). However, CTL recognition of a viral peptide copresented by two different MHC alleles of the same haplotype has not been reported to date. For example, the Sendai virus peptide SEV-9 (NP324–332) binds to the two H-2[b] alleles H-2D[b] and H-2K[b] (Deres *et al.,* 1992; Schumacher *et al.,* 1991), can generate an extremely diverse CTL response *in vivo* (Cole *et al.,* 1994), but is only recognized by CTL in the context of H-2K[b] (Cole *et al.,* 1994; Kast *et al.,* 1991, Schumacher *et al.,* 1991). Interestingly, *in vitro* studies have shown that a single peptide can indeed be presented to CTL by both H-2D[b] and H-2K[b] molecules (Sheil *et al.,* 1994).

Do H-2K[b] and H-2D[b] separately restrict the LCMV GP1 epitope? Since MHC molecules directly influence antigen processing (Falk *et al.,* 1990; Kozlowski *et al.,* 1993; Malarkannan *et al.,* 1995; Ojcius *et al.,* 1994) and consequently the CTL response, an important point to address was the independence (vs H-2D[b]) and the efficiency of the anti-GP1, H-2K[b]-restricted CTL response. Dissection of the H-2K[b]-restricted GP1-specific T-cell response did not reveal significantly different CTL activity patterns in the presence or absence of H-2D[b] expression (see Figs. 4a, 4b, 4d, and 4e). Furthermore, the phenomenon of H-2 restriction (also illustrated in Figs. 4f and 4h) showed unambiguously that anti-GP1 H-2K[b]-restricted CTL did not recognize the viral antigen presented in the context of H-2D[b] restriction, and conversely. Thus, the H-2K[b]- and H-2D[b]-restricted CTL responses raised against the GP1 epitope in acutely infected mice are mounted separately, are efficient in the two contexts, and involve independent sets of MHC-restricted CTL. One can consider that the GP1 epitope behaves as two structurally distinct antigens in the two contexts. Indeed, TCR recognition of a viral antigen occurs not only via discrete contacts but also via an antigenic molecular surface of the peptide/MHC complex (Garboczi *et al.,* 1996; Garcia *et al.,* 1996; Quaratino *et al.,* 1995). It is likely that the antigenic surfaces of the GP1/H-2K[b] and GP1/H-2D[b] complexes in contact with the TCR markedly differ. Interestingly, 3 of 8 polymorphic residues of the MHC α1 and α2 domains which are accessible to the TCR and differ in H-2D[b] and H-2K[b] molecules have been described as TCR contacts (Ajitkumar *et al.,* 1988; Sun *et al.,* 1995). In addition, the possibility that most of the residues of the GP33–41 sequence (C-terminal anchor excepted) can serve as TCR contacts in one MHC context or the other, adds to the variability of the antigenic surface and potentially increases the diversity of the T cell repertoire.

Is GP1 the unique H-2K[b]-restricted immunodominant epitope of LCMV? Computer scanning of the LCMV protein sequences revealed that 18 other peptides harbored the H-2K[b] binding motif (Hudrisier and Gairin, unpublished observation) and might represent potential H-2K[b]-restricted epitopes. However, the observations that mutations within the known LCMV H-2[b]-restricted epitope sequences abolished CTL recognition and did not generate an efficient CTL response against another antigen (Lewicki *et al.,* 1995, 1995a; Oldstone *et al.,* 1995; and Fig. 7A) strongly suggested that none of these 18 peptides

represented an immunodominant H-2K^b-restricted epitope, favoring the hypothesis that GP34–41 is the unique immunodominant LCMV epitope in this context.

The finding that the GP1 epitope induces an efficient CTL response in both H-2D^b and H-2K^b contexts has important consequence in the understanding of the cellular immune response to LCMV infection. First, although the GP1 peptide does not match perfectly the MHC binding motif (Falk *et al.,* 1991; Gairin *et al.,* 1995) and exhibits the lowest MHC binding affinity among the known LCMV (Gairin *et al.,* 1995) and other H-2D^b viral epitopes (Hudrisier *et al.,* 1996), the increased repertoire diversity due to its dual allelic presentation may allow the GP1 epitope to generate an efficient primary and/or secondary CTL response in H-2^b mice following acute LCMV infection (Whitton *et al.,* 1988) or vaccination with the peptide itself (Aichele *et al.,* 1994, 1995; Kyburz *et al.,* 1993) or minigenes in vaccinia virus vectors (Klavinskis *et al.,* 1992). Second, the immunodominance and the efficiency of the H-2K^b-restricted component of the anti-GP1 CTL response may explain, at least in part, why acute LCMV infection is still controled after altering or blocking the H-2D^b-restricted component. This is well shown in RIP-LCMV NP transgenic mice that either coexpress adenoviral E3 gene (that prevents trafficking of H-2D^b but not H-2K^b) in β-cells (von Herrath *et al.,* 1997) or are treated with an H-2D^b-blocking peptide (von Herrath *et al.,* 1997a). In these mice, virus is still cleared following LCMV challenge, presumably by the H-2K^b component of the anti-CTL response. Interestingly, the observation that these mice do not develop diabetes following LCMV challenge very likely reflects the absence of H-2K^b-restricted anti-NP CTL response.

Dual MHC allelic presentation of the same viral antigen to CTL also has a profound incidence on host–virus interaction and CTL escape strategies. Many pathogens escape CTL recognition by single mutation of an epitope residue (Franco *et al.,* 1995). The relevance *in vivo* of antigenic variants for escape strategies is still controversial, and whether or not mutations in the sequence of an antigen presented by multiple MHC give an advantage to the host to avoid viral escape strategies (Moskophidis and Zinkernagel, 1995; Sidney *et al.,* 1995; and our study) remains to be determined. Natural LCMV GP1 variants that escape CTL recognition are known (Lewicki *et al.,* 1995; Moskophidis and Zinkernagel, 1995; Oldstone *et al.,* 1995; Pircher *et al.,* 1990). The fact that polyclonal populations of H-2^b CTL (bulk splenocytes from C57BL/6 mice) were used in these studies strongly suggests that these variants escape CTL lysis in both H-2K^b and H-2D^b contexts. We have reported previously that H-2D^b-restricted CTL pressure places one of these mutations (Phe^{38} → Leu^{38}) at a key residue of the GP1 sequence (Lewicki *et al.,* 1995, 1995a). Our present data support the hypothesis that, in this variant, the viral strategy to escape CTL lysis combines two different mechanisms: one that hampers MHC presentation (in the context of H-2K^b) (see Fig. 7B) and the other that very likely affects TCR recognition (in the context of H-2D^b). This result was predictable from molecular modeling studies (see Fig. 6).

By what mechanism does the GP1 epitope bind to H-2D^b and H-2K^b? A peptide can bind to multiple MHC molecules due to overlapping MHC motifs (Colbert *et al.,* 1994; Delguercio *et al.,* 1995; Fruci *et al.,* 1994; Kikuchi *et al.,* 1996; Luescher *et al.,* 1996; Sidney *et al.,* 1995; Tanigaki *et al.,* 1994) or by different mechanisms (Deres *et al.,* 1992; Matsumura *et al.,* 1992; Schumacher *et al.,* 1991). The binding of the LCMV GP1 epitope to H-2D^b and H-2K^b is due to the adjacent location of the H-2D^b and H-2K^b main anchors (Asn^{37} and Phe^{38}, respectively) in the peptide sequence, a structural feature similarly observed for the ovalbumin epitope OVA256-264 (ESIINFEKL) (Matsumura *et al.,* 1992). Further, the molecular models (see Fig. 6) illustrated how the core of the CTL epitope (recognition peptide) adopted markedly different conformations in the two MHC grooves, indicating that flexibility of the peptide backbone and adaptability of its side chains to a given binding groove are also important structural factors necessary for multiple MHC binding. The optimal H-2K^b binding sequence of the LCMV GP1 epitope (the decameric peptide GP34–43, AVYNFATCGI) is in accord with the expected anchor residues Tyr and Phe at positions 3 and 5, respectively (Rammensee *et al.,* 1995) but does not fit the canonical length of 8 residues which is the most commonly observed for H-2K^b-restricted antigens (Matsumura *et al.,* 1992; Rammensee *et al.,* 1995). In fact, it has been shown that the preferred size of H-2K^b-restricted peptides is sequence-dependent (Deres *et al.,* 1992) and peptides longer than 8 a.a. have been characterized that bound to H-2K^b (Joyce *et al.,* 1994; Schumacher *et al.,* 1991). The presence at the C-terminus of a residue not able to serve as MHC anchor (Gly^{42} for instance) resulted in decreased binding properties, an effect more pronounced on H-2D^b, as expected from previous studies (Gairin *et al.,* 1995; Matsumura *et al.,* 1992). Interestingly, a very good selectivity for H-2K^b was confered to the epitope by removal of the N-terminal Lys^{33} residue whereas, in contrast, only a very weak (if any) H-2D^b selectivity could be obtained (all the GP1 sequences tested bound almost equally well to H-2D^b and H-2K^b). This latter data helps explain previous observations showing that the H-2D^b binding properties of the GP1 epitope were affected by H-2K^b expression (Gairin *et al.,* 1995). In terms of CTL recognition, the octameric sequence GP34–41 (AVYNFATC) optimally sensitized target cells to H-2K^b-restricted CTL lysis, despite not showing the highest H-2K^b binding affinity. This lack of correlation between MHC binding and CTL recognition reflected that the C-terminal dipeptide Gly^{42}-Ile^{43}, although enhancing the MHC binding properties, was useless for CTL recognition in both contexts (H-2D^b and H-2K^b). This confirmed that non-preferred MHC binding peptides can be efficiently presented to CTL (Chen *et*

al., 1994). These findings further raise the questions of how the GP1 epitope is processed from the signal sequence of the virus and which exact sequence(s) is(are) naturally presented by H-2D^b and H-2K^b to CTL at the surface of LCMV-infected H-2^b cells. Their identification, based on the characterization of peptides eluted from MHC molecules, is currently under investigation in our laboratories.

In summary, the work presented in this study open up issues on the incidence and role of the dual allelic presentation of CTL epitope. The subject has been defined in context of LCMV Arm GP1 epitope and more particularly of its H-2K^b-restricted component on the pathogenesis of LCMV infection. It will be interesting to follow the pathological mechanisms of viral persistence and of CTL escape strategies in H-2D$^{b-/-}$ or H-2K$^{b-/-}$ knock-out transgenic H-2^b mice. It is also noteworthy that the H-2D^b-, H-2K^b-restricted LCMV GP1 epitope contains within its sequence the H-2K^d binding motif and could represent a subdominant H-2^d-restricted epitope (van der Most *et al.,* 1996). *In toto* these observations provide support for the concept that CTL epitopes may form clusters (Tussey *et al.,* 1995; Wilson *et al.,* 1996) within a viral protein sequence. In terms of therapeutic application, such single, minimal viral sequences that encompass overlapping CTL epitopes represent candidates of prime interest for the design and the development of anti-viral, peptide- or DNA-based vaccination strategies.

ACKNOWLEDGMENTS

The authors thank Drs. H. Mazarguil and B. Monsarrat for synthesis and mass spectrum analysis of LCMV peptides, Dr. J.-P. Abastado (Institut Pasteur, Paris) for the gift of the VSV NP52-57 peptide, and H. Lewicki for excellent technical assistance. This work was supported in part by Grants AG04342, JDF/DK995005, and AI09484.

REFERENCES

Aichele, P., Kyburz, D., Ohashi, P., Odermatt, B., Zinkernagel, R. M., Henartner, H., and Pircher, H. (1994). Peptide-induced T-cell tolerance to prevent autoimmune diabetes in a transgenic mouse model. *Proc. Natl. Acad. Sci. USA* 91, 444–448.

Aichele, P., Brduscha-Riem, K., Zinkernagel, R. M., Henartner, H., and Pircher, H. (1995). T cell priming versus T cell tolerance induced by synthetic peptide. *J. Exp. Med.* 182, 261–266.

Ajitkumar, P., Geier, S. S., Kesari, K. V., Borriello, F., Nakagawa, M., Bluestone, J. A., Saper, M. A., Wiley, D. C., and Nathenson, S. G. (1988). Evidence that multiple residues on both the alpha-helices of the class I MHC molecule are simultaneously recognized by the T cell receptor. *Cell* 54, 47–56.

Barber, L. D., and Parham, P. (1994). The essence of epitopes. *J. Exp. Med.* 180, 1191–1194.

Bergmann, C. C., Tong, L., Cua, R. V., Sensintaffar, J. L., and Stohlman, S. A. (1994). Cytotoxic T cell repertoire selection—A single amino acid determines alternative class I restriction. *J. Immunol.* 152, 5603–5612.

Byrne, J. A., Ahmed, R., and Oldstone, M. B. A. (1984). Biology of cloned cytotoxic T lymphocytes specific for lymphocytic choriomeningitis virus. *J. Immunol.* 133, 433–439.

Chen, Y., Sidney, J., Southwood, S., Cox, A. L., Sakaguchi, K., Henderson, R. A., Appella, E., Hunt, D. F., Sette, A., and Engelhard, V. H. (1994). Naturally processed peptides longer than nine amino acid residues bind to the class I MHC molecule HLA-A2.1 with high affinity and in different conformations. *J. Immunol.* 152, 2874–2881.

Colbert, R. A., Rowlandjones, S. L., Mcmichael, A. J., and Frelinger, J. A. (1994). Differences in peptide presentation between B27 subtypes: The importance of the P1 side chain in maintaining high affinity peptide binding to B*2703. *Immunity* 1, 121–130.

Cole, G. A., Hogg, T. L., and Woodland, D. L. (1994). The MHC class I-restricted T cell response to Sendai virus infection in C57BL/6 mice: a single immunodominant epitope elicits an extremely diverse repertoire of T cells. *Int. Immunol.* 6, 1767–1775.

Delguercio, M. F., Sidney, J., Hermanson, G., Perez, C., Grey, H. M., Kubo, R. T., and Sette, A. (1995). Binding of a peptide antigen to multiple HLA alleles allows definition of an A2-like supertype. *J. Immunol.* 154, 685–693.

Deres, K., Schumacher, T. N. M., Wiesmuller, K. H., Stevanovic, S., Greiner, G., Jung, G., and Ploegh, H. L. (1992). Preferred size of peptides that bind to H-2K^b is sequence dependent. *Eur. J. Immunol.* 22, 1603–1608.

Falk, K., Rotzschke, O., and Rammensee, H. G. (1990). Cellular peptide composition governed by major histocompatibility complex class I molecules. *Nature* 348, 248–251.

Falk, K., Rotzschke, O., Stevanovic, S., Jung, G., and Rammensee, H. G. (1991). Allele-specific motifs revealed by sequencing of self-peptides eluted from MHC molecules. *Nature* 351, 290–296.

Franco, A., Ferrari, C., Sette, A., and Chisari, F. V. (1995). Viral mutations, TCR antagonism and escape from the immune response. *Curr. Opin. Immunol.* 7, 524–531.

Fruci, D., Greco, G., Vigneti, E., Tanigaki, N., Butler, R. H., and Tosi, R. (1994). The peptide-binding specificity of HLA-B27 subtype (B*2705) analyzed by the use of polyalanine model peptides. *Hum. Immunol.* 41, 34–38.

Fung-Leung, W. P., Kundig, T. M., Ngo, K., Panakos, J., De Sousa-Hitzler, J., Wang, E., Ohashi, P. S., Mak, T. W., and Lau, C. Y. (1994). Reduced thymic maturation but normal effector function of CD8$^+$ T cells in CD8 beta gene-targeted mice. *J. Exp. Med.* 180, 959–967.

Gairin, J. E., Mazarguil, H., Hudrisier, D., and Oldstone, M. B. A. (1995). Optimal lymphocytic choriomeningitis virus sequences restricted by H-2D^b major histocompatibility complex class I molecules and presented to cytotoxic T lymphocytes. *J. Virol.* 69, 2297–2305.

Garboczi, D. N., Ghosh, P., Utz, U., Fan, Q. R., Biddison, W. E., and Wiley, D. C. (1996). Structure of the complex between human T-cell receptor, viral peptide and HLA-A2. *Nature* 384, 134–141.

Garcia, K. C., Degano, M., Stanfield, R. L., Brunmark, A., Jackson, M. R., Peterson, P. A., Teyton, L., and Wilson, I. A. (1996). An $\alpha\beta$ T cell structure at 2.5 Å and its orientation in the TCR-MHC complex. *Science* 274, 209–219.

Hammerling, G. J., Rusch, E., Tada, N., Kimura, S., and Hammerling, U. (1982). Localization of allodeterminants on H-2K^b antigens determined with monoclonal antibodies and H-2 mutant mice. *Proc. Natl. Acad. Sci. USA* 79, 4737–4741.

Hudrisier, D., Mazarguil, H., Laval, F., Oldstone, M. B. A., and Gairin, J. E. (1996). Binding of viral antigens by Major Histocompatibility Complex H-2D^b molecules are restricted by dominant elements at peptide nonanchor residues: implications for selection and presentation of T cell epitopes. *J. Biol. Chem.* 271, 17829–17836.

Hudrisier, D., Mazarguil, H., Oldstone, M. B. A., and Gairin, J. E. (1995). Relative implication of peptide residues in binding to major histocompatibility complex class I H-2D^b: application to the design of high-affinity, allele-specific peptides. *Mol. Immunol.* 32, 895–907.

Jameson, S., and Bevan, M. (1992). Dissection of major histocompatibility complex (MHC) and T cell receptor contact residues in a Kb-restricted ovalbumin peptide and an assessment of the predictive power of MHC-binding motifs. *Eur. J. Immunol.* 22, 2663–2667.

Joyce, S., Kuzushima, K., Kepecs, G., Angeletti, R. H., and Nathenson, S. G. (1994). Characterisation of an incompletely assembled major histocompatibility class I molecule (H-2K^b) associated with unusually

long peptides: implications for antigen processing and presentation. *Proc. Natl. Acad. Sci. USA* 91, 4145–4149.

Kast, W. M., Roux, L., Curren, J., Blom, H. J. J., Voordouw, A. C., Meloen, R. H., Kolakofsky, D., and Melief, C. J. M. (1991). Protection against lethal Sendai virus infection by in vivo priming of virus-specific cytotoxic T lymphocytes with a free synthetic peptide. *Proc. Natl. Acad. Sci. USA* 88, 2283–2287.

Kikuchi, A., Sakaguchi, T., Miwa, K., Takayima, Y., Rammensee, H. G., Kaneko, Y., and Takiguchi, M. (1996). Binding of nonamer peptides to three HLA-B51 molecules which differ by a single amino acid substitution in the A-pocket. *Immunogenetics* 43, 268–276.

Klavinskis, L. S., Whitton, J. L., Joly, E., and Oldstone, M. B. A. (1992). Vaccination and protection from a lethal viral infection: Identification, incorporation and use of a cytotoxic T lymphocyte glycoprotein epitope. *Virology* 178, 393–400.

Kozlowski, S., Corr, M., Shirai, M., Boyd, L. F., Pendleton, C. D., Berzofsky, J. A., and Margulies, D. H. (1993). Multiple Pathways Are Involved in the Extracellular Processing of MHC Class-I-Restricted Peptides. *J. Immunol.* 151, 4033–4044.

Kyburz, P., Aichele, P., Speiser, D. E., Hengartner, H., Zinkernagel, R. M., and Pircher, H. (1993). T cell immunity after viral infection versus T cell tolerance induced by soluble viral peptides. *Eur. J. Immunol.* 23, 1956–1962.

Lewicki, H., Tishon, A., Borrow, P., Evans, C. F., Gairin, J. E., Hahn, K. M., Jewell, D. A., Wilson, I. A., and Oldstone, M. B. A. (1995). CTL escape viral variants .1. Generation and molecular characterization. *Virology* 210, 29–40.

Lewicki, H., von Herrath, M. G., Evans, C. F., Whitton, J. L., and Oldstone, M. B. A. (1995a). CTL escape viral variants .2. Biologic activity in vivo. *Virology* 211, 443–450.

Luescher, I. F., Romero, P., Kuznetsov, D., Rimoldi, D., Coulie, P., Cerottini, J.-C., and Jongeneel, C. V. (1996). HLA photoaffinity labeling reveals overlapping binding homologous melanoma-associated gene peptides by HLA-A1, HLA-A29 and HLA-B44. *J. Biol. Chem.* 271, 12463–12471.

Malarkannan, S., Goth, S., Buchholz, D. R., and Shastri, N. (1995). The role of MHC class I molecules in the generation of endogenous peptide/MHC complexes. *J. Immunol.* 154, 585–598.

Matsumura, M., Fremont, D. H., Peterson, P. A., and Wilson, I. A. (1992). Emerging principles for the recognition of peptide antigens by MHC class I molecules. *Science* 257, 927–934.

Missale, G., Redeker, A., Person, J., Fowler, P., Guilhot, H. S., Schlicht, J., Ferrari, C., and Chisari, F. V. (1993). HLA-A31 and HLA-w68-restricted cytotoxic T cell responses to a single hepatitis B virus nucleocapsid epitope during acute viral hepatitis. *J. Exp. Med.* 177, 751–762.

Moskophidis, D., and Zinkernagel, R. M. (1995). Immunobiology of cytotoxic T-cell escape mutants of lymphocytic choriomeningitis virus. *J. Virol.* 69, 2187–2193.

Mylin, L. M., Bonneau, R. H., Lippolis, J. D., and Tevethia, S. S. (1995). Hierarchy among multiple H-2^b-restricted cytotoxic T-lymphocytes epitopes within Simmian Virus 40 T antigen. *J. Virol.* 69, 6665–6677.

Ojcius, D. M., Langlade-Demoyen, P., Gachelin, G., and Kourilsky, P. (1994). Role for MHC Class I molecules in selecting and protecting high affinity peptides in the presence of proteases. *J. Immunol.* 152, 2798–2810.

Oldstone, M. B. A., Lewicki, H., Borrow, P., Hudrisier, D., and Gairin, J. E. (1995). Discriminated selection among viral peptides with the appropriate anchor residues: implications for the size of the CTL repertoire and control of viral infection. *J. Virol.* 69, 7423–7429.

Oldstone, M. B. A., Tishon, A., Geckeler, R., Lewicki, H., and Whitton, J. L. (1992). A common antiviral cytotoxic T-lymphocyte epitope for diverse major histocompatibility complex haplotypes: Implication for vaccination. *Proc. Natl. Acad. Sci. USA* 89, 2752–2755.

Oldstone, M. B. A., Whitton, J. L., Lewicki, H., and Tishon, A. (1988). Fine dissection of a nine amino acid glycoprotein epitope, a major determinant recognized by lymphocytic choriomeningitis virus class I restricted H-2D^b cytotoxic T lymphocytes. *J. Exp. Med.* 168, 559–570.

Ozato, K., Hansen, T. H., and Sachs, D. H. (1980). Monoclonal antibodies to mouse MHC antigens. II. Antibodies to the H-2L^d antigen, the product of a third polymorphic locus of the mouse major histocompatibility complex. *J. Immunol.* 125, 2473–2477.

Pircher, H., Moskophidis, D., Rohrer, U., Burki, K., Hengartner, H., and Zinkernagel, R. M. (1990). Viral escape by selection of cytotoxic T cell-resistant virus variants *in vivo*. *Nature* 346, 629–633.

Quaratino, S., Thorpe, C. J., Travers, P. J., and Londei, M. (1995). Similar antigenic surfaces, rather than sequence homology, dictate T cell epitope molecular mimicry. *Proc. Natl. Acad. Sci. USA* 92, 10398–10402.

Rammensee, H. G., Friede, T., and Stevanovic, S. (1995). MHC Ligands and peptide motifs: First listing. *Immunogenetics* 41, 178–228.

Ruppert, J., Sidney, J., Celis, E., Kubo, R. T., Grey, H. M., and Sette, A. (1993). Prominent role of secondary anchor residues in peptide binding to HLA-A2.1 molecules. *Cell* 74, 929–937.

Saito, Y., Peterson, P. A., and Matsumura, M. (1993). Quantitation of peptide anchor residue contributions to class-I major histocompatibility complex molecule binding. *J. Biol. Chem.* 268, 21309–21317.

Schulz, M., Aichele, P., Wollenweider, M., Bobe, F. W., Cardinaux, F., Hengartner, H., and Zinkernagel, R. M. (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.

Schumacher, T. N. M., DeBruijn, M. L. H., Bernie, L. N., Kast, W. M., Melief, C. J. M., Neefjes, J. J., and Ploegh, H. L. (1991). Peptide selection by MHC class I molecules. *Nature* 350, 703–706.

Sheil, J. M., Schell, T. D., Shepherd, S. E., Klimo, G. F., Kioschos, J. M., and Paterson, Y. (1994). Presentation of a horse cytochrome c peptide by multiple H-2b class I major histocompatibility complex (MHC) molecules to C57BL/6 and bml-derived cytotoxic T lymphocytes: Presence of a single MHC anchor residue may confer efficient peptide-specific CTL recognition. *Eur. J. Immunol.* 24, 2141–2149.

Shibata, K. I., Imarai, M., VanBleek, G. M., Joyce, S., and Nathenson, S. G. (1992). Vesicular somatitis virus antigenic octapeptide N52-59 is anchored onto the groove of the H-2K^b molecule by the side chains of three amino acids and the main-chain atoms of the amino-terminus. *Proc. Natl. Acad. Sci. USA* 89, 3135–3139.

Shirai, M., Pendleton, C. D., and Berzofsky, J. A. (1992). Broad recognition of cytotoxic T cell epitopes from the HIV-1 envelope protein with multiple class I histocompatibility molecules. *J. Immunol.* 148, 1657–1667.

Sidney, J., Delguercio, M. F., Southwood, S., Engelhard, V. H., Appella, E., Rammensee, H. G., Falk, K., Rotzschke, O., Takiguchi, M., Kubo, R. T., Grey, H. M., and Sette, A. (1995). Several HLA alleles share overlapping peptide specificities. *J. Immunol.* 154, 247–259.

Sun, R., Sheperd, S. E., Geier, S. S., Thomson, C. T., Sheil, J. M., and Nathenson, S. G. (1995). Evidence that the antigen receptors of cytotoxic T lymphocytes interact with a common pattern on the H-2K^b molecule. *Immunity* 3, 573–582.

Tanigaki, N., Fruci, D., Vigneti, E., Starace, G., Rovero, P., Londei, M., Butler, R. H., and Tosi, R. (1994). The peptide binding specificity of HLA-B27 subtypes. *Immunogenetics* 40, 192–198.

Tishon, A., Lewicki, H., Rall, G., von Herrath, M., and Oldstone, M. B. A. (1995). An essential role for type 1 interferon-g in terminating persistent viral infection. *Virology* 212, 244–250.

Tussey, L. G., Rowland-Jones, S., Zheng, T. S., Androlewicz, M. J., Creswell, P., Frelinger, J. A., and McMichael, A. J. (1995). Different MHC class I alleles compete for presentation of overlapping viral epitopes. *Immunity* 3, 65–77.

van der Most, R., Sette, A., Oseroff, C., Alexander, J., Murali-Krishna, K., Lau, L. L., Southwood, S., Sidney, J., Chesnut, R. W., Matloubian, M., and Ahmed, R. (1996). Analysis of cytotoxic T cell responses to dominant and subdominant epitopes during acute and chronic lymphocytic choriomeningitis virus infection. *J. Immunol.* 157, 5543–5554.

von Herrath, M. G., Efrat, S., Oldstone, M. B. A., and Horwitz, M. S.

(1997). Expression of adenoviral E3 transgenes in β-cells prevents autoimmune diabetes. *J. Clin. Invest.,* submitted.

von Herrath, M. G., Gairin, J. E., Coon, B., and Oldstone, M. B. A. (1997a). A specific MHC class I restricted "blocking peptide" prevents activation of virus-induced CTL and the development of virus induced autoimmune diabetes. *Immunity,* submitted.

Whitton, J. L., Southern, P. J., and Oldstone, M. B. A. (1988). Analysis of the cytotoxic T lymphocyte responses to glycoprotein and nucleoprotein components of lymphocytic choriomeningitis virus. *Virology* 162, 321–327.

Wilson, C. C., Kalams, S. A., Wilkes, B. M., Ruhl, D. J., Gao, F., Hahn, B. H., Hanson, I. C., Luzuriaga, K., Wolinsky, S., Koup, R., Buchbinder, S. P., Johnson, R. P., and Walker, B. D. (1996). Overlapping epitopes in human immunodeficiency virus type 1 gp120 presented by HLA A, B, C molecules: effects of viral variation on cytotoxic T-lymphocyte recognition. *J. Virol.* 71, 1256–1264.

Young, A. C. M., Zhang, W. G., Sacchettini, J. C., and Nathenson, S. G. (1994). The 3-dimensional structure of H-2D^b at 2.4 angstrom resolution - Implications for antigen-determinant selection. *Cell* 76, 39–50.

Zhang, W. G., Young, A. C. M., Imarai, M., and Nathenson, S. G. (1992). Crystal structure of the major histocompatibility complex class I H-2K^b molecule containing a single viral peptide: implications for peptide binding and T-cell receptor recognition. *Proc. Natl. Acad. Sci. USA* 89, 8403–8407.