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Immunobiology of Cytotoxic T-Cell Escape Mutants of Lymphocytic Choriomeningitis Virus

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Infection with virus variants exhibiting changes in the peptide sequences defining immunodominant determinants that abolish recognition by antiviral cytotoxic T cells (CTL) presents a considerable challenge to the antiviral T-cell immune system and may enable some viruses to persist in hosts. The potential importance of such variants with respect to mechanisms of viral persistence and disease pathogenesis was assessed by infecting adult mice with variants of lymphocytic choriomeningitis virus (LCMV) strain WE. These variants were selected in vivo or in vitro for resistance to lysis by $CD8^+$ $H\text{-}2^b$ -restricted antiviral CTL. The majority of anti-LCMV CTL in infected $H\text{-}2^b$ mice recognize epitopes defined by residues 32 to 42 and 275 to 289 (epitopes 32–42 and 275–289)of the LCMV glycoprotein or 397 to 407 of the viral nucleoprotein. The 8.7 variant exhibits a change in the epitope 32–42 (Val-35→Leu), and variant CL1.2 exhibits a change in the epitope 275–289 (Asn-280→Asp) of the wild-type LCMV-WE. The double-mutated 8.7-B23 variant had the variation of 8.7 and an additional change located in the epitope 275–289 (Asn-280→Ser). The 8.7 variant peptide with unchanged anchor positions bound efficiently to $H\text{-}2D^b$ and $H\text{-}2K^b$ molecules but induced only a very weak CTL response. CTL epitope 275–289 of CL1.2 and 8.7-B23 altered at predicted anchor residues were unable to bind D^b molecules and were also not recognized by antiviral CTL. Infection of C57BL/6 mice ($H\text{-}2^b$) with the variants exhibiting mutations of one of the CTL epitopes, i.e., 8.7 or CL1.2, induced CTL responses specific for the unmutated epitopes comparable with those induced by infection with WE, and these responses were sufficient to eliminate virus from the host. In contrast, infection with the double-mutated variant 8.7-B23 induced CTL activity that was reduced by a factor of about 50-fold compared with wild-type LCMV. Consequently, high doses (10^7 PFU intravenously) of this virus were eliminated slowly and only by about day 100 after infection. 8.7-B23 failed to cause lethal lymphocytic choriomeningitis after intracerebral infection with a dose of $>10^4$ PFU in C57BL/6 mice (but not in mice of nonselecting $H\text{-}2^d$ haplotype); with the other variants or wild-type LCMV, doses greater than 10^6 to 10^7 PFU were necessary to avoid lethal choriomeningitis. Thus, CTL escape mutations in a noncytopathic virus, even in only some but not of all CTL epitopes, significantly modulate the virus-host relationships and disease patterns in an major histocompatibility complex-specific and dose-dependent fashion.

T lymphocytes recognize foreign peptides in association with molecules of the major histocompatibility complex (MHC), using a diverse repertoire of T-cell receptors (TCR). These TCR are αβ heterodimers generated by rearrangement of variable, diversity, and joining region gene segments during thymocyte development (58). T-cell responses in an individual to a foreign protein from either a viral or a nonviral source is generally restricted to a few but some times only one immunodominant peptides derived by processing of the antigen (25, 61). Various reasons have been suggested for this limitation, including availability of an appropriate peptide after antigen processing by specific intracellular proteases, specificity of peptide-MHC interactions, competition from other peptides, antagonistic activity of peptides, and limitations of the expressed T-cell repertoire (7, 20, 25). Lymphocytic choriomengitis (LCMV), a noncytopathic RNA virus, induces in the mouse, its natural host, a protective cytotoxic T-cell (CTL) response that causes both elimination of the virus and immunopathological disease (3, 11, 14, 22, 44, 64). The majority of LCMV-specific $CD8^+$ CTL in mice of $H\text{-}2^b$ haplotype recognize peptides defined by residues 32 to 42 or 275 to 289 of the viral glycoprotein (GP) (CTL epitope 32–42 or 275–289), presented by K^b and D^b or D^b MHC molecules, respectively, and residues 397 to 407 of the viral nucleoprotein (NP), presented by D^b MHC molecules (24, 34, 47, 55). These represent 50 to 60%, 10 to 20%, and 20 to 30% of the total CTL activity, respectively.

There is evidence indicating that noncytopathic or poorly cytopathic viruses may mutate epitopes recognized by CTL and thereby escape CTL recognition and control (for reviews, see references 36 and 41). Infection with virus variants with particular changes in the peptide sequences defining immunodominant determinants may therefore modify the virus-host relationships in an MHC-dependent fashion. We evaluated this issue by defining distinct CTL epitope mutants of LCMV-WE and studying the $CD8^+$ CTL response in C57BL/6 mice infected with these variants. The results of this study reveal limitations in the plasticity of an antiviral CTL immune response. They suggest that infection with virus variants that are resistant to specific CTL or emergency of such variants in situ may modulate the immune response to viruses, facilitating a prolonged persistence of the infectious agent and possibly leading to chronic immunopathological disease in the host.

MATERIALS AND METHODS

Animals. C57BL/6 ($H\text{-}2^b$) and BALB/c ($H\text{-}2^d$) mice were obtained from the Institut für Zuchthygiene (University of Zurich, Switzerland).

Cells and culture conditions. The generation, maintenance, and specificity of the CTL clones 50.1 and B23.35 (B23), which are specific for the T-cell epitope defined by residues 275 to 289 of the LCMV GP [LCMV-GP-(275–289)] have

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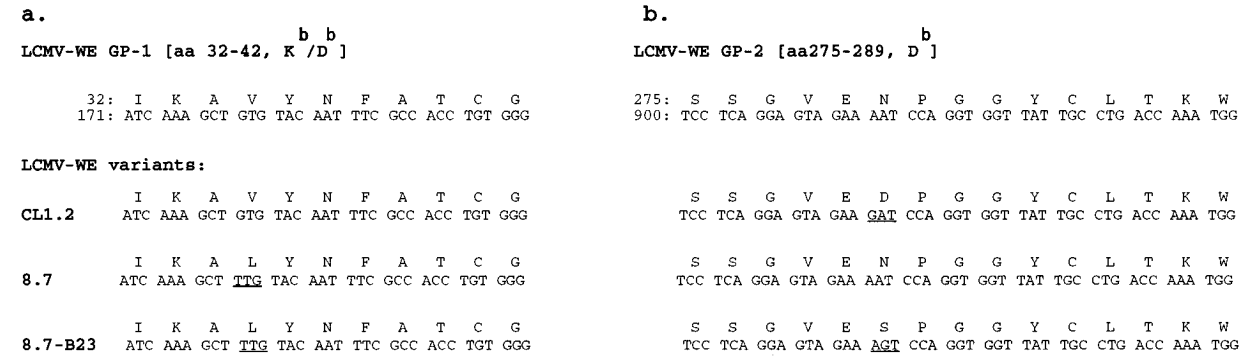

FIG. 1. Characterization of LCMV-WE CTL-resistant variants exhibiting point mutations in *H-2*^{b} CTL epitopes. Nucleotide and deduced amino acid (aa) sequences, of the CTL epitopes 32–42 and 275–289 of the viral glycoprotein of LCMV-WE and its variants are shown. The nucleotide sequence is given in viral sense (57). Differences the nucleotide sequence are underlined.

been described in detail elsewhere (2, 6, 52). The αβ TCR clone was established from a C57BL/6 mouse carrying the transgenic TCR α and β chains specific for the LCMV-GP-(32–42) (53). Polyclonal CTL specific for the T-cell epitope defined by residues 32 to 42 or 275 to 289 of the LCMV GP or for residues 397 to 407 of the LCMV NP were obtained by restimulation of memory spleen cells from virus-infected C57BL/6 mice with macrophages loaded with the corresponding peptides (Neosystem Laboratories, Strasbourg, France). Primary antiviral splenic CTL were prepared as single-cell suspensions from animals infected intravenously (i.v.) with 100 PFU of the LCMV wild-type strain WE or the variant isolates. Cells used as target cells were MC57G (*H-2*^{b}) or D2 (*H-2*^{d}).

Selection of virus variants. The WE strain of LCMV was originally obtained by F. Lehmann-Grube (Hamburg, Germany). Stock virus was grown on L929 cells from a triple-purified virus. Virus titers were determined on L929 cell monolayer cultures (38) or with an immunological focus assay (8). The generation and characterization of LCMV-8.7, a variant obtained from LCMV-specific TCR-transgenic mice infected with 10^6 PFU of LCMV-WE, has been described previously (55). LCMV-WE variants CL1.2 and 8.7-B23 were selected in vitro by using a procedure described previously (1). Briefly, MC57G or B6-SV40 cells (3×10^5 cells per well) were seeded into six-well tissue culture plates and infected with LCMV at multiplicities of infection of 1 to 0.01, CTL (2×10^6 to 3×10^6) were added 1 to 4 h later, and the supernatants containing virus were collected after 48 h, titrated, and tested in a standard cytotoxicity assay. Virus isolates were cloned twice or three times by plaque purification as described previously (56). LCMV-CL1.2 was isolated from cell cultures infected with LCMV-WE in the presence of polyclonal CTL specifically recognizing the CTL epitope defined by residues 275 to 289 of the LCMV GP. The 8.7-B23 variant of LCMV-WE was obtained by infecting cells in vitro with LCMV-8.7 together with the CTL clone B23, which recognizes the CTL epitope defined by residues 275 to 289 of the LCMV GP (52).

Detection of antiviral CTL activity in the spleen of mice infected with LCMV-WE or virus variants. The frequencies of antiviral CTL precursor cells (CTLp) were determined under limiting-dilution conditions as described in detail previously (43). The antiviral CTL activities were determined in a standard ^{51}Cr release assay for 4 to 5 h. MC57G cells were infected with LCMV-WE of its variants at multiplicities of infection of 0.001 to 0.2 for 2 days or were loaded with peptides for 2 h. The peptide of LCMV-WE or variants defined by residues 32 to 42 (IKAVYNFATCG or IKALYNFATCG) or 275 to 289 (SSGVENPGGY CLTKW, SSGVEDPGGYCLTKW, or SSGVESPGGYCLTKW) of the LCMV GP or residues 397 to 407 (QPQNGQFIHFY) of the LCMV NP (Neosystem Laboratories) were used at 100 μM for coating of the target cells. Effector cells were clones or polyclonal CTL specific for particular viral CTL epitopes. The primary CTL activity in the spleens of virus-infected mice was expressed as lytic units per spleen; a lytic unit was defined as the number of splenocytes required to lyse one-third of a standard number of target cells (10^4 cell per well) (46).

Quantification of infectious virus in tissues of infected mice. The quantification of infectious virus in the spleen and kidney of mice infected with a low (10^2 PFU i.v.) or high (10^7 PFU i.v.) does of LCMV-WE or of variants has been described previously (38).

Sequence analysis of LCMV-WE variants. LCMV-WE selectants (8.7, CL1.2, and 8.7-B23) were purified from cell culture supernatants by using procedures described previously (49). Viral RNA was isolated by digestion with proteinase K, phenol-chloroform extraction, and sequential precipitation with ethanol. Viral RNA (2 μg) was then used as a template for DNA synthesis by reverse transcriptase (Moloney murine leukemia virus; Pharmacia Uppsala, Sweden), using the manufacturer's conditions and the following specific primers: I (5′-TCG TAGCATTGTCACAGAATTCTTC-3′), complementary to nucleotides 1014 to 1037 of the viral S-RNA and amplifying the region including the CTL epitope defined by residues 32 to 42 and 275 to 289 of the LCMV GP; II (5′-GAGAG GCCTCAAGCTTCTGGAAGT-3′), complementary to nucleotide 2945 to 2967 of the viral S-RNA and amplifying the region of the viral nucleoprotein including the CTL epitope defined by residues 397 to 407; and III (5′-GATCCGAAGTC TACATCAAAG-3′), complementary to nucleotides 3061 to 3081 of the viral S-RNA and amplifying the region of the LCMV NP including the CTL epitope defined by residues 118 to 132.

The cDNA was amplified by PCR using *Taq* polymerase (Boehringer, Mannheim, Germany) with primers I and IV (5′-GAATTCTATCCAGTAAAAG GATGG-3′; complementary to nucleotides 58 to 81 of the viral S-RNA), with primers II and V (5′-GGGAGAGCACCTATAACTGATGAGG-3′; complementary to nucleotides 1976 to 2000 of the viral S-RNA), or with primers III and VI (5′-CCCTTACTACACCACTTGC-3′; complementary to nucleotides 2841 to 2859 of the viral S-RNA). PCR was run for 42 cycles on a thermal cycler (Perkin-Elmer Cetus Corp., Emeryville, Calif.). Denaturation was for 1 min at 94°C, and extension for 3 min at 72°C. The PCR product was isolated from the agarose gel by using a Millipore filter (QTY UF OHV25 ultrafree MC; 0.45-mm pore size; Pharmacia, Uppsala, Sweden) and sequenced with a *Taq* dye-deoxyterminator cycle sequencing kit on an Applied Biosystem 373A DNA sequencing system (Foster City, Calif.) according to the manufacturer's conditions. To avoid PCR artifacts, viral RNA was sequenced from at least four independent virus preparations. In addition, both strands were sequenced by using suitable primers. Sequences were compared to those for WE described previously (57).

RESULTS

Characterization of LCMV variants exhibiting *H-2*^{b} restricted CTL epitope escape mutations. Nucleotide sequence analysis of the viral variants in regions of the viral S-RNA containing the CTL epitopes in mice of *H-2*^{b} haplotype revealed amino acid changes within the relevant epitopes in comparison with wild-type WE (Fig. 1). The 8.7 variant showed a point mutation (Val-35→Leu) within CTL epitope 32–42; CL1.2 showed a point mutation (Asn-280→Asp) within CTL epitope 275–289. The 8.7 B23 variant exhibited the mutation of 8.7 in CTL epitope 32–42 (Val-35→Leu) and an additional one (Asn-280→Ser) within CTL epitope 275–289. We did not find additional variations in the deduced amino acid sequences of the viral S-RNA fragments that we have sequenced, i.e., nucleotides 60 to 380, 620 to 1020, and 1970 to 2210, including the relevant CTL epitopes of *H-2*^{b} haplotype, and 2840 to 3060, containing epitope 118–132 of the viral NP in mice of *H-2*^{d} haplotype.

The LCMV-WE variants were analyzed further in a cytotoxicity assay, using as effector cells either clones or polyclonal CTL specific for particular epitopes of LCMV-WE (Fig. 2). The 8.7 and 8.7-B23 variants were not recognized by the αβ TCR-transgenic CTL clone, and polyclonal CTL specific for epitope 32–42 of LCMV-WE showed greatly (>10 times) reduced cytotoxicity. The recognition of epitope 275–289 of variants CL1.2 and 8.7-B23 by specific CTL was completely abolished. Target cells (MC57G) infected with these variants were as susceptible as LCMV-WE-infected target cells to lysis by

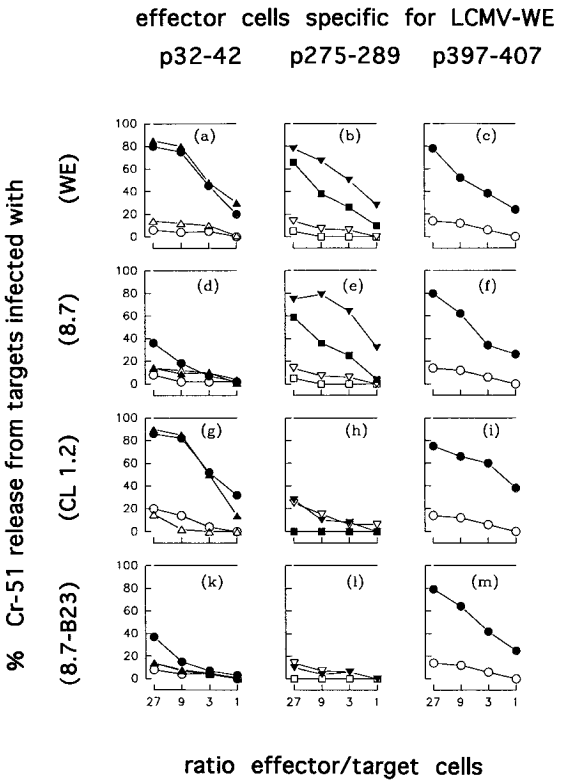

FIG. 2. Susceptibility of MC57G (*H-2*^{b}) target cells infected with the variants of LCMV-WE to lysis by CTL lines or clones specific for the indicated epitopes of LCMV-WE. Target cells were infected (filled symbols) with LCMV-WE (a to c), 8.7 (d to f), CL1.2 (g to i), and 8.7-B23 (k to m) or uninfected (open symbols). Effector cells were polyclonal CTL recognizing specifically the epitope 32–42 (●, ○; a, d, g, and k) or 275–289 (▼, ▽; b, e, h, and l) of the GP or 397–407 (●, ○; c, f, i, and m) of the NP of LCMV-WE. In addition, the target cells were tested with CTL clones specific for peptide 32–42 originating from LCMV-specific TCR-transgenic mice (▲, △; a, d, g, k) and or for peptide 275–289 (clone B23; ■, □; b, e, h, and l).

CTL specific for the respective unchanged epitopes. In addition, polyclonal CTL from BALB/c mice (*H-2*^{d}) specifically recognized epitope 118–132 and efficiently lysed D2 target cells infected with LCMV-WE or its variants (data not shown). Further experiments were performed to evaluate whether LCMV-WE variants were able to induce CTL specific for their variant peptides. For this purpose, splenocytes from C57BL/6 mice infected i.v. with 100 PFU of the variants of WE were tested on day 8 to 10, periods of maximum CTL activity, using MC57G target cells infected with virus or labelled with peptides of the CTL epitopes of LCMV-WE or of variants. The data confirmed the absence of CTL activities specific for the variant peptides of CL1.2 and 8.7-B23 at CTL epitope 275–289 and considerably reduced activity for the peptide of 8.7 variant at epitope 32–42 (Table 1 and data not shown). These observations were extended in experiments using CTL obtained by restimulation several times in vitro with variant peptides of splenocytes from C57BL/6 mice infected with the mutants; CTL activities specific for variant peptide 275–289 of CL1.2 or 8.7-B23 were not detectable. The peptide of 8.7 induced CTL which recognized and lysed target cells labelled with variant peptide 32–42 or infected with 8.7 virus efficiently as has been reported previously (54). The majority of these CTL recognized the variant 8.7 epitope presented by K^b and less by D^b MHC molecules (data not shown).

The impaired ability of variants to induce an antiviral CTL

TABLE 1. Capacities of LCMV-WE and variants to induce primary CTL specific for variant epitopes in infected mice

Target cells[a]	Effector/target cell ratio	Effector CTL induced against LCMV[b]			
		8.7	C1.1.2	8.7-B23	WE
LCMV	70	71, 58	93, 76	55, 49	85, 88
	23	40, 40	59, 51	38, 29	46, 45
	8	23, 19	33, 34	11, 14	28, 32
8.7 (peptide 32–42) (Val-35→Leu)	70	11, 14	8, 11	8, 14	6, 10
	23	2, 6	2, 4	2, 6	2, 3
	8	0, 4	0, 3	0, 3	2, 2
CL1.2 (peptide 275–289) (Asn-280→Asp)	70	2, 3	4, 2	3, 1	4, 2
8.7-B23 (peptide 275–289) (Asn-280→Ser)	70	3, 2	1, 3	4, 2	3, 3
Unlabelled targets	70	2, 3	4, 2	3, 4	3, 5

[a] Target cells were infected with LCMV-WE or its variants and tested with corresponding virus-specific CTL. Alternatively, target cells were labelled with the indicated peptide.

[b] Ability of CTL induced by infection of C57BL/6 mice with 100 PFU of LCMV-WE or its variant to lyse MC57G (*H-2*^{b}) target cells infected with LCMV or labelled with viral peptides. Values represent percentages of ^{51}Cr release at the indicated effector-to-target cell ratio in a 4- to 5-h CTL assay. Results were obtained from two experiments.

that specifically recognized variant or wild-type virus epitopes may reflect either inability in presentation of peptides by virus-infected cells due to lack of binding to MHC class I molecules or alternatively failure of T cells to recognize the altered peptides. We tested binding of variant peptides by studying up-regulation of MHC molecules (K^b or D^b) on RMA-S cells treated with different concentrations of wild-type or variant peptides. The experiments reveal that a change at residue Asn-280 to Asp (CL1.2) or Ser (8.7-B23) prevents binding and presentation of the variant peptide by D^b molecules. The variation Val-35→Leu (8.7) did not affect binding to MHC molecules but rather influenced recognition by D^b-restricted specific CTL (data not shown). This peptide stabilized *H-2D*^{b} or *H-2K*^{b} on the surface of RMA-S cells to a similar extent as the corresponding peptide of LCMV-WE, as has been reported previously (5). Thus, alteration at predicted anchor residues within CTL epitope 275–289 of CL1.2 and 8.7 B23 variants affects binding to MHC molecules and prevents development of epitope-specific antiviral CTL. In contrast, the non-anchor residue variation of 8.7 within CTL epitope 32–42 did not affect presentation of the peptide but considerably impaired development of specific antiviral CTL.

Impaired antiviral CTL response against CTL escape mutant viruses in vivo. To assess whether accumulation of CTL epitope mutations influenced the capacity of the host to develop an adequate CTL response, able to control LCMV infection in various tissues, C57BL/6 mice were infected with the LCMV variants. The dose of the viral inoculum (10^2 or 10^7 PFU) or route of infection (i.v. or intracerebrally [i.c.]) was varied. Intracerebral inoculation of LCMV usually causes a lethal lymphocytic choriomeningitis (LCM) (37).

LCMV-WE variants induced distinct levels of antiviral CTL activity in C57BL/6 mice (Fig. 3). The double mutant 8.7-B23 induced a considerably lower level of overall antiviral CTL activity compared with the variants with one altered CTL epitope or wild-type WE. This correlated with reduced cytotoxicity for CTL epitope 32–42 and absence of specific activity for epitope 275–289. CL1.2 and 8.7 variants induced comparable levels of CTL activity despite the absence or reduced

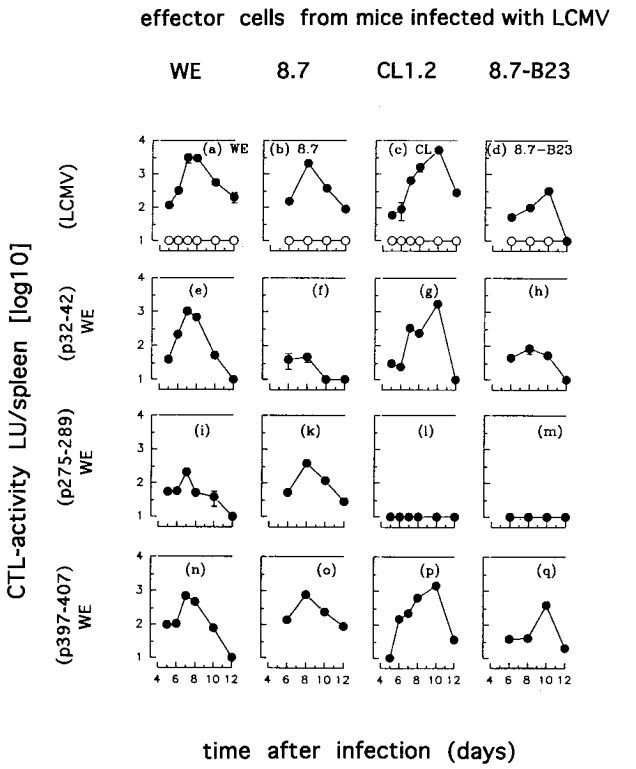

FIG. 3. Primary CTL activity in the spleens of C57BL/6 mice infected with LCMV-WE or variants. Mice were infected i.v. with 100 PFU of the LCMV indicated at the top, and the CTL activity was determined on MC57G target cells infected with the various LCMV isolates (a to d) or loaded with LCMV-WE peptide 32–42 (e to h) or 275–289 (i to m) of the GP or 397–407 (n to q) of the NP. Antiviral CTL activity was expressed as lytic units (LU) per spleen. Datum points represent means ± standard errors of the means of 5 to 10 mice; in most cases, standard errors of the means are less than the size of the symbol used. Filled symbols, target cells virus infected or labelled with peptides; open symbols, untreated target cells.

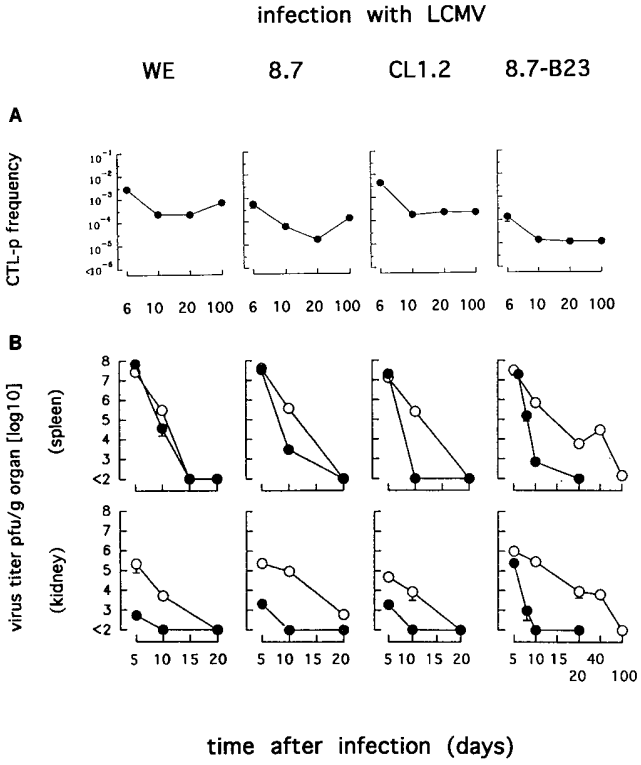

FIG. 4. Antiviral CTLp activity and kinetics of virus replication versus elimination of LCMV-WE variants in C57BL/6 mice. Mice were infected i.v. with 10^7 PFU of the LCMV indicated at the top. The CTLp activity in the spleen was assessed by limiting dilution and is indicated as frequency (A). Datum points represent means ± standard errors of the means of four to five mice. In addition, virus titers were determined in the spleens or kidneys of C57BL/6 mice infected i.e. with a relatively low dose of 10^2 PFU (filled symbols) or a high dose of 10^7 PFU (open symbols) of LCMV-WE or its variants (B). Datum points represent means ± standard errors of the means of 5 to 10 mice.

CTL activity for epitopes 275–289 and 32–42, respectively (Fig. 3).

The ability of C57BL/6 mice to eliminate CTL-resistant variants from different tissues was tested in mice infected i.v. with a relatively low dose (10^2 PFU) or high dose (10^7 PFU) of the virus. Virus titers were determined in the spleen and kidney during a period of 100 days after the infection (Fig. 4B). At low doses, the viruses were eliminated within a period of 2 weeks. It is noteworthy that higher (about 100 times) virus titers were detected on day 5 after infection in the kidneys of mice infected with a low dose of double-mutated virus than in kidneys of mice infected with other variants or WE. Infection with 10^7 PFU caused persistence of the 8.7-B23 variant in the tested tissues for up to 100 days. Elimination of variants 8.7 and CL1.2 variants was complete by day 20 except for variant 8.7 in kidneys. The capacity of C57BL/6 mice to eliminate LCMV has been correlated in an earlier study with the relative frequencies of antiviral CTLp (46). The levels of antiviral CTLp during induction and during the memory phase were therefore determined in the mice infected with 10^7 PFU (or 10^2 PFU; data not shown) of LCMV-WE or the variants (Fig. 4A). The frequencies of antiviral CTLp induced after infection with CL1.2 did not differ from those of mice infected with the wild-type WE. Infection with 8.7 initially led to extensive expansion of CTLp (days 6 to 10); they rapidly declined to a low level around day 20 and later increased again to a level comparable to that observed on day 10 of infection. In general, the CTLp memory levels induced by 8.7 were three- to fivefold lower than those found after infection with WE. In contrast, a long-lasting profound reduction of CTLp was observed in mice infected with 8.7-B23 up to day 100; the frequencies of CTLp were about 50-fold lower than those after infection LCMV-WE. The profound transient reduction of CTLp on day 20 after infection with the 8.7 or 8.7-B23 variant is reminiscent of the exhaustion of antiviral CTLp after infection with LCMV-Docile or C1 13-Armstrong (46). This reduction was absent in mice infected with a low dose of 10^2 PFU i.v. (data not shown).

Inoculation i.c. of a relatively low dose of 10^2 PFU of LCMV usually induces a characteristic lethal LCM, mediated preferentially by antiviral CTL (6, 14, 27). Inoculation of a relatively high dose ($>10^6$ PFU) has been shown to protect mice sometimes from lethal LCM (high-dose immune paralysis) (30, 50). To assess the role of variations within viral CTL epitopes in the development of disease mediated by antiviral CTL, we tested the LCMV-WE variants for the ability to cause high-dose immune paralysis. LCMV-WE or CL1.2 virus injected i.c. at 10^6 or 10^7 PFU per mouse spared about 75% of inoculated C57BL/6 mice. In contrast, 8.7 virus failed to cause lethal LCM when injected i.c. at 10^5 PFU per mouse. The LCMV-8.7-B23 variant caused high-dose immune paralysis most readily; a relatively low dose of 10^3 to 10^4 PFU i.c., but not higher doses, was lethal for about 50% of the infected mice. The effect of the CTL epitope mutations on susceptibility of LCM was specifi-

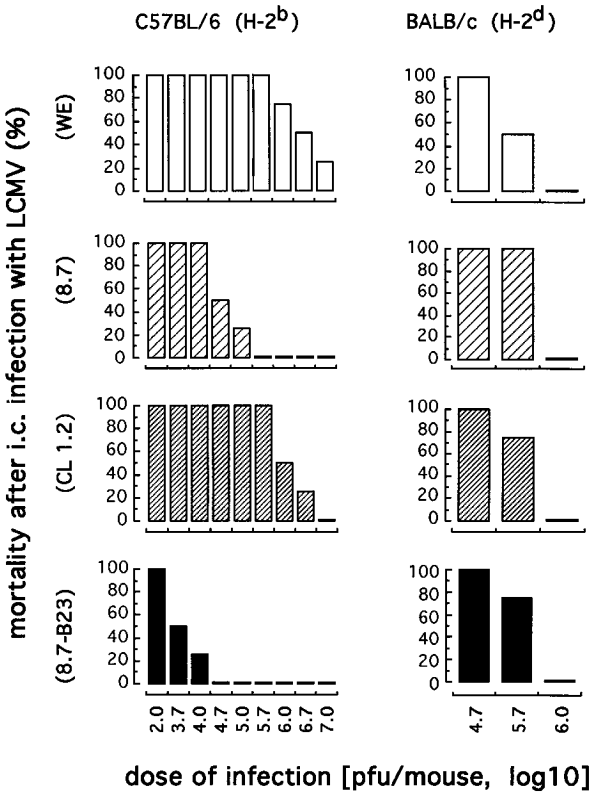

FIG. 5. Comparisons of susceptibility to lethal LCM of mice infected with *H-2*^{b} CTL epitope escape LCMV variants. C57BL/6 (*H-2*^{b}) or BALB/C (*H-2*^{d}) mice were infected i.c. with increasing doses of LCMV-WE or variants. Development of lethal LCM in experimental groups of 10 to 15 mice was monitored for a period of 30 days. Column represent percentages of mortality of three experiments combined.

cally linked to the selecting *H-2*^{b} MHC haplotype because infection i.c. of BALB/c (*H-2*^{d}) mice with the variants or WE wild-type virus revealed comparable susceptibilities to LCM (Fig. 5).

DISCUSSION

This report provides evidence that CTL escape variant viruses that have mutated one to two but not all three CTL epitopes reduce the overall CTL response and therefore may facilitate persistence of the noncytopathic virus in the host; thus, even partial CTL escape of noncytopathic viruses may change the virus-host relationships and severity of immunopathological disease significantly. LCMV variants which escape from recognition by antiviral CTL have been defined by mutations within the amino acid sequence of the immunodominant CTL epitope in mice of the MHC *H-2*^{b} haplotype. The relevant escape mechanisms occur as a result of alterations in anchor residues affecting peptide binding (mutation within epitope, 275–289 of CL1.2 and 8.7-B23 virus) or in non-anchor residues affecting recognition (mutation within epitope 32–42 of 8.7 or 8.7-B23). In the first case, the change in the anchor position (N→D in CL1.2 or N→S in 8.7-B23) prevents binding by D^b and therefore also recognition by T cells. In the latter case, the variant peptide binds to D^b and K^b MHC molecules and will be recognized by variant peptide-specific CTL restricted preferentially to K^b molecules, but overall it does not induce a pronounced CTL response to that epitope in vivo. Whether this reflects similarity to a self-peptide, defect in the TCR repertoire, or another mechanism remains unclear. As tested so far in vitro, the 8.7 peptide does not act as a competitor for the corresponding wild-type WE peptide (3a) and does not block expression of MHC class I.

Accumulation of mutations in various CTL epitopes in the noncytopathic LCMV gradually changes the antiviral CTL response of the host. Infection with variants possessing alterations in one of the three *H-2*^{b}-restricted CTL epitopes did not impair CTL responses considerably. The excess of antiviral CTL activity directed to unchanged epitopes enabled recovery from the viral infection. In contrast, infection with a variant carrying substitutions within two of three CTL epitopes modified its host relationship significantly. This variant replicated widely in different tissues of the mouse and induced a reduced CTL response; this resulted in persistent viral infection and prevented immunological disease in the host. These results suggest several questions. Why can mutations in two but not all CTL epitopes have such a significant effect on the virus-host relationship? What is the survival advantage of such variants in the infected host? To what extent may CTL escape variants, either preexisting in the infectious inoculum or emerging in situ, contribute to create conditions which permit viral persistence? Can such variants spread though a population of individuals differing in MHC type?

The finding that mutated virus with mutations in two major CTL epitopes on GP but possessing an unaltered third epitope in NP suggests that changes that may be considered to be relatively small influence the biological equilibrium between virus and host immune system significantly. This does not come as too much of a surprise, since relatively subtle differences between LCMV isolates in susceptibility to alpha, beta, or gamma interferon, in replication kinetics, or in tropism, in addition to several other host factors, including *H-2* (4, 45, 48), influence the host-virus equilibrium significantly.

Recent investigations have provided some insights into how relative immunodominance in the class I-restricted CTL responses may be explained. Although the affinity of MHC class I-peptide binding clearly has a role in selecting determinants recognized by CTL, proteolytic mechanisms involved in processing of antigen, peptide stability, peptide transport, and the CTL repertoire may also account for immunodominance (7, 13, 21, 42, 59). The limited available data, however, cannot yet explain the hierarchical distribution of antiviral CTL reactivities in *H-2*^{b} mice, postulating differences in affinities of peptide-MHC *H-2D*^{b} or *-K*^{b} interactions. In addition to these discussed factors, clonal deletion by exhaustion may be enhanced by CTL escape mutations and change overall kinetics of both virus and CTL responses to prevent lethal immunopathology, which is the parameter used as a main readout in this study. Unfortunately, a precise quantification is not yet possible for most of these parameters.

To avoid recognition by the host's CTL response, viruses use various mechanisms such as (i) antigen mimicry (viral peptides presented by class I MHC molecules may mimic self-peptides) (12). (ii) variations in MHC molecules that alter presentation of particular CTL epitopes (17, 18, 41), (iii) viral interference with T-cell recognition by down-regulation of MHC class I expression or of proteins that alter or block presentation of viral peptides by MHC class I molecules (28, 40), and finally (iv) selection of CTL-resistant variants. The latter case would seem to be a precarious situation in protective immunity to infectious pathogens that frequently mutate and change antigens. Variations in the sequence of the viral genome may affect CTL recognition by different ways: by blocking correct transport and processing of the antigen, blocking binding to MHC

molecules, or blocking or impairing recognition of the peptide-MHC complex by TCR (10, 15, 18, 19, 23, 31, 35, 39, 62). The existence of CTL escape mutant viruses has now been documented and studied in detail for influenza virus (23). Epstein-Barr virus (17, 18), LCMV (1, 55), human immunodeficiency virus (15, 16, 32, 33, 35, 51, 60) and hepatitis B virus (9, 10). In the latter two examples, not only T-cell epitope escape mutants but also antagonostic viruses that exhibit epitopes that seem to be able to anergize CTL of the original specificity have been found (10, 35). It is noteworthy that CTL escape mutants have been described for viruses that are genetically unstable and that tend to persist in the host. It would not be to the selective advantage of a rapidly lethal, lytic virus to emphasize this mechanism. These studies collectively reveal that mutations within the viral genome causes them to evade recognition by CTL through a variety of mechanisms and suggest that the cumulative action of various escape variants may modulate the virus-host relationship toward persistence of a noncytopathic virus. The clear impact of *H-2* on susceptibility to LCM in general, as shown earlier (4, 48, 50), and of sequence variations affecting *H-2*^{b}-restricted CTL activities on the virus-host relationships and phenotype of disease illustrates that the relative number of available CTLp is rather limited in the host and has a direct influence on these parameters. This has also been demonstrated in a comparative study infecting BALB/c (*H-2*^{d}) and BALB/c *H-2*^{dm2} mutant mice with LCMV-Docile or C1 13-Armstrong. The BALB/c *H-2*^{dm2} mice are not able to present the CTL epitope defined by residues 118 to 132 of the LCMV NP because of lack of presenting L^d MHC molecules and consequently mount a weak response specific for the LCMV GP (references 4, 26, and 29), and unpublished observations). Virus clearance was drastically impaired in the mutant mice, and the susceptibility to CTL exhaustion was vastly increased (4, 45, 63).

What are the consequences of the shift in relative CTL kinetics caused by elimination of one or two of three relevant CTL epitopes? As has been point out before and clearly documented here, such mutations could be relevant only for a host of a particular MHC class I configuration. Somewhat surprisingly, the data from this report suggest the probability that even partial CTL escape may have significant effects on the virus-host relationship. Because the CTL response during a viral infection is usually polyclonal and polyspecific, this renders emergence of variants, predominating within the replicating virus, difficult but not impossible. Infection with variants lacking one or more immunodominant epitopes may create the condition for further selection of CTL escape mutants in the infected host. Furthermore, accumulation of variants with an array of mutations affecting several CTL epitopes may represent reservoirs of highly virulent viruses for a broad population of individuals across several MHC haplotypes. Although direct evidence for the implication of CTL escape mutants with respect to mechanisms of viral persistence in the broad context of viral epidemiology are rare and indirect, there are interesting examples that have recently come to light; first, mutations in epitopes of the human immunodeficiency virus protein Gag, which are presented by HLA-B8, have been found and are not recognized by CTL present in the same patient (51); second, loss of HLA A11-restricted CTL epitopes in Epstein-Barr virus isolates from A11-positive populations by selective mutations of anchor residues has been reported (17); third, selection of CTL escape variants in a subset of patients with chronic hepatitis B virus infection who express a narrow repertoire of anti-hepatitis B virus CTL has been suggested to contribute to viral persistence (9). Therefore, it is possible that during the course of infection with noncytopathic viruses, variant viruses that become increasingly less recognizable by CTL in hosts of differing MHC class I types may emerge and thereby change the subtle virus-host relationships and disease patterns.

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