Relationship Among Immunodominance of Single CD8+ T Cell Epitopes, Virus Load, and Kinetics of Primary Antiviral CTL Response

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Relationship Among Immunodominance of Single CD8 $^+$ T Cell Epitopes, Virus Load, and Kinetics of Primary Antiviral CTL Response$^1$

Gunnar Weidt,$^2$ Olaf Utermöhlen, Jochen Heukeshoven, Fritz Lehmann-Grube,$^3$ and Wolfgang Deppert

The primary CTL response of BALB/c mice infected with the lymphocytic choriomeningitis (LCM) virus strain WE is directed exclusively against one major epitope, n118, whereas a viral variant, ESC, that does not express n118 induces CTL against minor epitopes. We identified one minor epitope, g283, that induces primary lytic activity in ESC-infected mice. Infections of mice with WE and ESC were used to study the hierarchical control of a T cell response. Presentation of minor epitopes is not reduced in WE-infected cells. Generation of CTL against n118 does not suppress the generation of minor epitope-specific CTL systemically, as mice coinfected with WE and ESC developed CTL against n118 and g283. However, elimination of ESC and development of minor epitope-specific CTL in ESC infection were slower than elimination of WE and development of CTL against n118. CD8$^+$ T cells against the minor epitope were activated in ESC and WE infection, but did not expand in the latter to show lytic activity in a primary response. We explain the absence of minor epitope-specific lytic activity in WE infection by the fast reduction of virus load due to the early developing n118-specific CTL. Immunodominance of CTL epitopes in primary virus infections thus can be explained as a kinetic phenomenon composed of 1) expansion of CD8$^+$ T cells specific for individual epitopes, 2) stimulatory effect of virus load, and 3) negative feedback control on virus load by the fastest CTL population. The Journal of Immunology, 1998, 160: 2923–2931.

Elimination of viruses essentially depends on the response of CD8$^+$ T cells to peptide epitopes presented by MHC class I molecules. The CTL response against viruses is often focused on a few epitopes. The reason for this selectivity is not obvious; many peptides were identified in viral proteins by scanning for MHC class I binding motifs that are able to bind to MHC class I molecules (1–3). However, despite being able to bind, only some of these peptides are recognized in naturally induced CTL responses. Different mechanisms that prevent the use of such peptides as T cell epitopes in vivo might be considered. At the level of Ag processing, generation of peptides could be inefficient due to sequence specificity of degradation by the proteasome; transport of the peptide into the endoplasmic reticulum might be affected due to sequence selectivity of the TAP transporter system (4–6). This could result in insufficient presentation of peptides with binding affinity to MHC class I molecules for activating CTL precursors, thereby preventing a detectable CTL response. Finally, competition of different peptides for limited transporter capacity or MHC class I binding (7) could also prevent presentation. At the T cell level, “holes” in the T cell repertoire due to limited T cell precursor frequency (8–10), or peptide-specific tolerance (11) were recognized as reasons for nonresponsiveness of CD8$^+$ T cells against certain viral peptides presented by MHC class I molecules.

There is also evidence for a dynamic control of the CTL response at the T cell level. In the absence of the immunodominant epitope, a formally subdominant epitope can take over a dominant function. In this respect, immunodominance is not a static property, but describes the relative CTL responses to two or more epitopes in a given immunologic context. To avoid conceptual difficulties in describing the relative strength of T cell epitopes, we here use the terms major and minor epitopes.

A well-studied example is the hierarchical control of the CTL response against T Ag in SV40-transformed cells. The CTL response against T Ag in C57BL/6 (B6) mice is directed against three major class I-restricted T cell epitopes. CTL against a minor epitope are only obtained by immunization with a variant T Ag that does not express the major epitopes. Generation of minor epitope-specific CTL is not caused by enhanced presentation of the minor epitope. Major and minor epitopes do not compete for presentation (12, 13). The absence of CTL against the minor epitope in wild-type T Ag-injected mice thus must be regulated at the T cell level. Similarly, a hierarchy of epitopes was observed during infection of mice with LCM$^4$ virus. C57BL/6 mice infected with CTL escape variants of LCM virus, which do not express the defined major epitopes, generate CTL against minor epitopes, not recognized by primary CTL during infection with wild-type virus (14). In BALB/c mice, the primary CTL response against LCM virus is directed against one major epitope in the viral nucleoprotein (NP), amino acids (aa) 118 to 126 (n118), presented by the

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$^2$ Address correspondence and reprint requests to Dr. Gunnar Weidt, Center for Immunotherapy of Cancer, University of Connecticut, 263 Farmington Ave., Farmington, CT 06030-1601. E-mail address: weidt@uke.uni-hamburg.de

$^3$ Deceased, July 8, 1996. This paper is dedicated to his memory.

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4 Abbreviations used in this paper: LCM, lymphocytic choriomeningitis; NP, nucleoprotein; aa, amino acids; pfu, plaque-forming units; GP, glycoprotein.
MHC class I molecule Ld (15, 16). In the BALB/c mutant C-H-2dm2 a compensatory primary antiviral CTL response arises against epitopes that are restricted by Kd and Dd (17), since these mice cannot create n118-specific CTL due to loss of the MHC class I molecule Ld (18).

We investigated the mechanism of hierarchical control of T cell responses in the acute infection of BALB/c mice with LCM virus, strain WE, or a LCM virus variant (ESC) described previously (19). Infection with the viral variant ESC that does not express the dominant epitope n118 induces a CTL response against minor CD8+ T cell epitopes that are not recognized by primary CTL that develop during WE infection. In this report we describe experiments supporting the mechanism of a kinetic control of CTL generation, which prevents the development of a primary lytic activity against minor epitopes when major epitopes are present. This mechanism could explain the hierarchy of CTL epitopes and the adaptability of the CTL response.

Materials and Methods

Virus and mice

LCM virus strain WE (20) and the WE-derived variant ESC (19) were propagated and titrated as pfu in L929 cells as previously described (21). Recombinant vaccinia viruses expressing the LCM viral GP (Vacc-NP6) or glycoprotein (GP; Vacc-G2) were propagated and titrated in VERO cells (22, 23).

BALB/cAnNcrlBR, C57BL/6crlBR (B6), and [BALB/cAnNcrlBR × C57BL/6crlBR]F1 (CB6) mice were purchased from Charles River Wiga (Sulzfeld, Germany). All mice were kept under specific pathogen-free conditions in the animal facility of the Heinrich Pette Institute. Female mice were used when they were 8 to 12 wk old.

Protein peptides, vaccines, and immunization

Nonapeptides corresponding to potential LCM viral T cell epitopes were predicted by allele-specific motifs (2). Peptides for immunization and sensitizing target cells were synthesized by continuous flow F-moc strategy as previously described (24). Eight peptides from the viral GP were synthesized and purified by reverse phase HPLC using a common acetonitrile/trifluoroacetic acid/water system: g7 (aa 7–15), g30 (aa 30–38), g35 (aa 35–43), g264 (aa 264–272), g283 (aa 283–291), g314 (aa 314–322), g342 (aa 342–350), and g348 (aa 348–356). Peptide n118 corresponds to the dominant epitope and represents the LCM-viral NP aa 118–126. Mice were immunized s.c. or i.p. with 5 μg of protein together with 0.5 μg of SDS in 0.5 ml of PBS 7 days before being infected with 103 or 106 pfu of LCM virus.

PCR analysis of virus population

Ratios of WE to ESC in virus populations were determined by restriction site polymorphism as previously described (19). Briefly, a mutation changed glutamine to arginine in the major epitope n118 at position 3 of the epitope and simultaneously destroyed the function of the epitope and the HidIII restriction site in the cDNA of WE. RNA from L cells infected for 44 h with virus from spleens of infected mice, with a virus stock of WE, or ESC, or with both was harvested (27) and reverse transcribed. A fragment of the viral NP spanning a region containing the epitope was amplified by PCR. Products were incubated with HindIII, and cleavage was analyzed by agarose gel electrophoresis.

Chromium release assay

Primary CTL activity of splenocytes was determined by a 4.5-h standard assay performed as previously described (28) with modifications (17). Assays were performed on day 8 of infection if not stated otherwise. SV40-transformed BALB/c and C57BL6 (B6) fetal fibroblasts were used as target cells for H-2d- and H-2b-restricted CTL, respectively. For H-2k-restricted CTL, L929 cells were used as targets. Target cells were infected with LCM virus WE or ESC 48 h before the assay. Alternatively, cells were incubated for 30 min with 10−7 M of the indicated nonapeptide.

Results

Identification of one minor H-2Kd-restricted CTL epitope in the viral GP

The primary CTL activity of BALB/c mice during LCM virus infection with strain WE is predominantly directed against the major epitope n118 of the viral NP. We previously described a CTL escape variant (ESC) that does not express the major epitope. ESC virus-infected BALB/c mice generate a primary CTL response against minor MHC class I-restricted T cell epitopes, not recognized by primary CTL in response to WE infection (19). At least one minor epitope is probably located in the viral GP, because BALB/c mice were protected against ESC infection by immunization with recombinant vaccinia virus coding for the viral GP (Table I). Vaccinia virus coding for NP was protective against WE, but protection was very low against ESC infection. We therefore conclude that NP does not contain suitable minor epitopes.

![Table II. Protective capacity of fragments of viral glycoprotein and epitope vaccines containing n118 or g283 in immunizing BALB/c mice against infection with LCM virus strain WE](http://www.jimmunol.org/.Download)
with these peptides. Only mice injected with peptide g283 showed significantly reduced virus titers (4 × 10^3 vs 2 × 10^2 pfu (WE), or 3.8 × 10^3 vs 3.1 × 10^2 pfu (ESC)/g of spleen) on day 5 of infection. Immunization with the other peptides did not reduce virus titers significantly (data not shown). Protection against WE infection was obtained by immunization with epitope vaccines containing the major epitope n118, as described previously (26). Also the epitope vaccine T-g283, consisting of the SV40 T-Ag fragment (aa 2–270) and the minor LCM virus epitope g283, protected BALB/c mice against LCM virus infection (Table II, Expt. II). The efficiency of protection induced by T-g283 was slightly reduced compared with that of T-n118, containing the major epitope. Peptide g283 represents a minor epitope, because in repeated experiments ESC-infected as well as g283-sensitized target cells were lysed to the same extent by ESC-specific CTL (Fig. 1). Importantly, the generation of g283-specific CTL during ESC infection is not simply caused by an enhanced presentation of g283, as WE- or ESC-infected or g283-loaded BALB/c fibroblast target cells were lysed to the same extent by primary CTL from ESC-infected mice. This indicates that presentation of g283 by the MHC class I molecule H-2Kd is not affected by the presentation of the major H-2Ld-restricted epitope n118 in WE-infected cells in vitro. The same holds true in vivo, since WE-infected as well as ESC-infected mice were equally protected by immunization with peptide g283, epitope vaccine T-g283, or GP265–494 containing this epitope (see above; data not shown). We therefore conclude that the observed hierarchy of the major n118 over the minor g283 epitope in the immune response of LCM virus-infected mice must be regulated at the T cell level.

The CTL response against minor epitopes is not suppressed by systemic factors during infection with WE

Suppression of the response against minor T cell epitopes by the response against the major epitope by systemic factors has been discussed to explain the hierarchy of T cell epitopes. It was suggested that the T cell response against the minor epitopes is suppressed by the developing T cell response specific for the major epitope by unknown, possibly soluble, factors (12). To investigate the possibility of such systemic effects in our system, we infected BALB/c mice with WE, ESC, or both viruses and measured the primary CTL activity specific for major and minor epitopes. As shown in Figure 1A, the lytic activity of CTL from WE-infected mice is directed predominantly against n118, seen on WE-infected or peptide n118-sensitized target cells. Little activity was seen on target cells infected with ESC or loaded with peptide g283. The pattern of CTL reactivity was completely different in ESC virus-infected mice. No lysis specific for n118 was observed; instead, CTL against g283 and possibly other minor epitopes emerged. Compared with single infections, the lytic activity against g283 was reduced in coinfections when the virus mixture contained 90% ESC in the injected virus mixture. This confirms that the T cell response against the minor g283 epitope is not suppressed by systemic factors in vivo.

If minor epitope-specific CTL were suppressed in WE-infected mice by systemic factors induced by the major epitope n118-specific CTL, this suppression should also occur in mice coinfected with WE and ESC. This, however, was not the case, because in coinfection we observed primary lytic activity against n118 as well as against g283 (Fig. 1A). Compared with single infections, the lytic activity of CTL specific for n118 and more pronounced for g283 was reduced in coinfection. We investigated this reduction in detail by injecting mixtures with different ratios of WE and ESC. As shown in Figure 1B, lytic activity against g283 was already reduced in coinfections when the virus mixture contained >10% WE, whereas reduction of n118-specific activity required as much as 90% ESC in the injected virus mixture. This confirms that individual CTL responses against both WE and ESC coexisted in the

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**FIGURE 1.** CTL response to major and minor T cell epitopes of BALB/c mice infected with LCM virus WE or ESC or with mixtures of both. A, Primary CTL activity against minor and major CTL epitopes, specifically against n118 and g283, in spleens of BALB/c mice infected i.v. with 10^7 pfu of WE (open squares), ESC (open triangles), or both (closed circles). Target cells were BALB/c fibroblasts infected with WE or ESC or loaded with peptides corresponding to epitopes n118 and g283. Specific lysis of uninfected and unlabeled target cells was <5%. B, Effect of coinfection with different ratios of WE and ESC on the distribution of CTL activity of spleen lymphocytes against n118-labeled (closed squares) and g283-labeled (closed circles) target cells. Two mice per group received a total of 200 pfu by the indicated mixture of WE and ESC 8 days before the CTL assay. Equal amounts of spleen cells were mixed; an E:T cell ratio of 100:1 is shown.
same mouse and demonstrates that only the extent of the CTL responses against the major and the minor epitope was influenced by the ratio between WE and ESC in coinfection in a dose-dependent manner.

**Minor epitope-specific CTL are activated during WE infection, but expand inefficiently**

Next we asked whether minor epitope-specific lytic activity during WE infection is prevented by insufficient activation or by failure of expansion of minor epitope-specific CD8$^+$ T cells. During LCM infection, it is difficult to differentiate between activation and expansion due to the fast expansion of virus-specific CD8$^+$ T cells. However, epitope-specific activation of CD8$^+$ T cells with very little expansion can be achieved by vaccination with a nonreplicating epitope vaccine. Recombinant protein vaccines are presented by professional APC, such as dendritic cells and macrophages (30, 31), and activate CD8$^+$ T cells as described previously (26). Expansion of epitope-specific activated T cells then can be induced by challenging the immunized mice with virus. However, a high dose infection of 10$^6$ pfu of virus is necessary to measure the primary CTL response, since it is not possible to detect such a response after low dose infection with 10$^2$ pfu. The failure to measure a primary CTL response after infection with 10$^2$ pfu of virus in immunized mice can be explained by a limited stimulation of T cell expansion due to the fast declining virus load (29). When mice are injected with 10$^6$ pfu WE without prior immunization, the CTL response is strongly reduced due to the phenomenon of high zone immune paralysis, as reported previously (32), and these mice developed only a low CTL response against WE-infected target cells (see Fig. 2A). The residual lytic activity observed on day 6 after high dose infection was directed against n118. Peptide-sensitized target cells were recognized more efficiently than WE-infected cells presenting endogenously produced n118, which may reflect the low affinity of n118-specific CTL after high dose infection.

The monoepitopic vaccines T-n118 and T-g283 activated CD8$^+$ T cells specific for the major or the minor LCM virus epitope, respectively. Activation was seen by an enhanced ex vivo CTL response against the corresponding epitope on day 6 after challenge infection with high virus doses (Fig. 2A). After immunization with T-n118, a CTL response specific for n118 was observed during challenge infection with WE or coinfection with WE and ESC (Fig. 2A and B), but not during challenge with ESC (data not shown). These findings reflect the fact that ESC does not encode a functional n118 and therefore cannot stimulate preactivated n118-specific T cells to expand. Following immunization with T-g283, the CTL response specific for g283 was observed regardless of the type of challenge virus. Next we asked whether CD8$^+$ T cells specific for g283 and n118 were activated when both monoepitopic vaccines were injected into the same mouse. CTL against n118 and g283 were observed simultaneously during cochallenge of such immunized mice with both viruses, indicating that activation of minor epitope-specific CTL was not suppressed by coadministration of the major and the minor epitope. Interestingly, when challenged with either WE or ESC, CTL specific only for n118 or g283, respectively, were found. In mice coimmunized with n118 and g283, g283-specific CTL did not expand following WE challenge infection. This effect was not mediated by systemic factors, because CTL with both specificities emerged during coinfection (Fig. 2). Essentially the same results were obtained regardless of whether both monoepitopic vaccines were coadministered i.p. or were injected separately into the left and the right flank s.c. to prevent their uptake by the same professional APC. This result indicated that T cells against the minor and the major epitope were activated in coimmunized mice, but that minor epitope-specific cells did not expand during challenge infection with WE when T cells against the major epitope n118 were present.

**Kinetics of WE and ESC clearance in BALB/c mice**

To explain these phenomena, we investigated whether WE or ESC virus were eliminated with different efficiencies by minor and major specific CD8$^+$ T cells. In a first approach we determined elimination of WE or ESC during high dose challenge infection after immunization with epitope vaccines. The virus load in T-n118-vaccinated mice on day 5 of high dose infection with WE was 100-fold lower compared with that in nonimmunized mice, whereas immunization with T-g283 reduced the virus load in WE- and ESC-infected mice only 10-fold (Table II, Expt. III). These data indicate that the antiviral activities of CD8$^+$ T cells specific for the major or the minor epitope at defined time points during infection with WE or ESC, respectively, were different. To explain the expansion of CD8$^+$ T cells directed exclusively against n118 after immunization against both n118 and g283 after WE challenge, we suggest that g283-activated CD8$^+$ T cells in WE challenge infection do not receive sufficient stimuli for expansion due to the fast elimination of WE by n118-specific antiviral CD8$^+$ T cells.

In a second approach, we analyzed the virus load during the course of low dose infection (10$^5$ pfu) of naive mice with WE or ESC to investigate the stimuli mediated by the respective virus for the expansion of antiviral n118 and g283-specific CD8$^+$ T cells.
Both viruses replicate with the same efficiency in vivo, as seen by identical maximal virus titers on days 4 to 5 during infection of BALB/c mice (Table I and Fig. 3A). CD8+ T cell-mediated elimination of WE and ESC in BALB/c mice begins on day 5 of infection. However, elimination of ESC is delayed, as the virus load in the spleen on days 6 and 7 in ESC-infected mice is 10 times higher than that in WE-infected mice. Mice coinfected with 10^2 pfu of each virus were infected with 44 h with virus from spleens of infected mice. PCR products corresponding to a fragment of viral NP containing epitope n118 were treated with HindIII and separated by agarose gel electrophoresis. For the control, L cells were infected with a multiplicity of infection of 0.001 of a cell culture-derived virus stock of WE, ESC, or equal amounts of both and incubated for 44 h before RNA was harvested.

**Hierarchy of T cell epitopes in F1 hybrid mice**

To date, our data suggested that in the system analyzed, the hierarchy of T cell epitopes depends on different kinetics of the development of CTL against major and minor epitopes. Thereby, the fastest CTL population exerted its negative effect on the expansion of minor epitope-specific CTL by rapidly reducing the virus load, i.e., by removing the stimulus for the expansion of major epitope-specific CTL. We asked whether this concept of kinetic control of CTL development can explain the relative strength of CTL responses during LCM virus infection not only for the major and minor BALB/c epitopes, but is generally applicable for other LCM virus epitopes present in mice of other haplotypes. Therefore, we extended our observations to LCM virus T cell epitopes in BALB/c mice.

We mated BALB/c (H-2b) mice with B6 mice (H-2b) and investigated the CTL response of the resulting CB6 F1 hybrid mice during infection with WE and ESC. B6 mice were chosen, since their CTL response against three codominant epitopes has been analyzed in detail. One of them, g33, corresponds to aa 33 to 41 of the viral GP (33, 34).

B6-specific lytic activity was assayed on WE-infected or peptide (corresponding to epitope g33)-sensitized H-2b fibroblasts; BALB/c-specific activity was measured on WE-infected or n118- or g283-sensitized H-2d fibroblasts. The primary CTL response in CB6 mice during infection with WE was directed against n118 as was against at least one of the H-2b-restricted dominant B6 epitopes, g33 (Fig. 5B). This indicates that the major H-2b epitope n118 and the major H-2b epitope g33 with regard to hierarchy are on the same level. CB6 mice infected with ESC generated primary CTL predominantly against (one or more) B6 epitopes (Fig. 5B). Obviously, only a low CTL activity against minor BALB/c epitopes, specifically g283, was observed in the presence of a CTL response against the major B6 epitopes. This indicates that g283 is a minor epitope in context with the major BALB/c epitope, n118, as well as in context with major B6 epitopes. In agreement with our hypothesis, the rapid elimination of virus in WE-infected BALB/c mice as well as in WE- or ESC-infected CB6 mice correlated with the absence of minor epitope-specific CTL. Primary lytic activity

**Kinetics of development of CTL specific for major and minor epitopes**

The faster clearance of WE compared with ESC suggested that CD8+ T cells against n118 develop more rapidly than CD8+ T cells against g283. To investigate the correlation between elimination of WE and ESC virus and the development of lytic activity against n118 and g283, we determined the time course of the epitope-specific CTL response in WE-infected, ESC-infected, and coinfected mice. As shown in Figure 4, CTL activity against n118 in WE-infected mice was detectable from days 6 to 10, with a peak of activity on day 8 of infection. The kinetics of CTL activity were identical in WE-infected and n118-sensitized target cells. No lytic activity on g283-loaded target cells was observed. ESC-infected mice did not show any CTL response specific for the epitope n118. As expected, the activity against the minor epitope g283 developed slower and was detected earliest on day 7 of infection, with a peak activity on day 10, confirmed in a kinetic experiment from days 8 to 14 (Fig. 4C and data not shown). CTL activity against minor epitopes decreased after day 10 of infection (data not shown). In coinfected mice, CTL activities against the major and the minor epitope developed independently with kinetics similar to those in single infections (Fig. 4, B and C).
against the minor epitope g283 was observed only in BALB/c mice infected with ESC, in which virus elimination is retarded compared with that in CB6 mice infected with ESC (Fig. 5) and the expansion of minor epitope-specific CTL was stimulated by a relatively high virus load.

**Discussion**

Dominance of CD8+ T cell epitopes was observed in the immune response during viral or bacterial infections in experimental and clinical systems. Immunodominance was discussed to be important for the development of protective immunity against viral infections and possibly also for the elimination of tumor cells. The distribution of the CTL responses against major and minor epitopes during HIV and HBV infection was investigated in detail and discussed with regard to selection of viral escape variants and consequences for pathogenesis (35–38). In acute and persistent EBV infections, the CTL response very often is focused against a single or a few epitopes (39). Selection of epitopes during the immune response was evaluated in acute infection of mice with influenza virus A (8, 10, 40–42) and LCM virus (14, 43). Immunodominance also is responsible for the failure of cross-protective immunity against different strains of *Theileria parva* in cattle due to induction of CTL against dominant strain-specific epitopes (44). The fundamental biochemical aspects of processing and presentation of epitopes and their contribution to immunodominance were investigated with murine cytomegalovirus (45, 46), and *Listeria monocytogenes* (5, 6).

However, not all aspects of the dominance of certain epitopes in an immune response can be explained by biochemical differences in Ag processing and presentation. It has been reported that the CTL response to complex Ags can adapt when the response against major epitopes is abrogated, supporting a hierarchy of major over minor epitopes (12, 17, 19). In these systems, CTL against minor epitopes developed if major epitopes were absent. Although we did not analyze the presentation biochemically, like other investigators, we have no evidence for an enhanced presentation of minor epitopes in the absence of major epitopes, which would simply explain the induction of CTL against minor epitopes (12, 44). Instead, regulation of CTL responses at the T cell level was suggested (12). One example for the adaptability of a CTL response, with a shift from a major to a minor epitope and generation of CTL against new epitopes without an influence of Ag processing,
was observed in BALB/c mice constitutively expressing the LCM viral NP in the thymus. These transgenic mice were partially tolerant against n118, could not generate high affinity CTL against the dominant n118, and developed a compensatory CTL response during LCM virus infection against epitopes in the viral GP (11).

CTL escape variants of tumor cells or viruses are powerful tools to investigate the phenomenon of immunodominance (13, 47, 48). Escape variants with alterations in dominant epitopes were described for the LCM virus in two haplotypes. In B6 mice (H-2b) a compensatory CTL response was observed during infection with escape variants, but the mechanism of hierarchical activation has not been investigated until now (14, 49, 50). We investigated the mechanism of T cell regulation leading to the observed adaptability of the CTL response and hierarchy of CTL epitopes during acute infection of BALB/c mice (H-2b) with LCM virus. We used the LCM virus strain WE and an escape variant (ESC) that does not express the dominant epitope n118. In the variant, the epitope was mutated, and the peptide corresponding to the mutated sequence is nonimmunogenic in BALB/c mice (19). Further, the variant is fully replication competent compared with the original virus strain, thereby providing an ideal system for analysis of the mechanism of immunodominance. ESC induced primary CTL against the minor epitope g283, as defined in this study. We found specific lysis of target cells sensitized with the peptide corresponding to g283 if infected with WE or ESC. g283 is one of two recently described minor LCM virus epitopes in BALB/c mice recognized by memory CTL after LCM virus wild-type infection (43).

The results reported here suggest a kinetic model for the adaptability of the T cell response regulated at the T cell level: CTL specific against major epitopes develop quickly and effectively reduce the virus load. The fast declining virus load then does not provide enough stimulus for the slower developing, minor epitope-specific CTL in a primary CTL response. If the fast expansion of CTL against the dominant epitopes is abrogated, the virus load drops more slowly and provides sufficient stimulus for the expansion of minor epitope-specific CTL. We suggest that kinetic differences in the expansion or maturation of CTL populations specific for major and minor epitopes can explain many observations regarding the hierarchy of CTL epitopes. We found remarkable differences in the kinetics of WE and ESC virus elimination and conclude that the observed fast decrease in WE virus load is due to rapidly developing CTL against n118. Thus, caused by insufficient stimulation by the fast declining virus load, expansion of slower CD8+ T cells directed against minor epitopes did not occur during WE infection to a measurable degree. In agreement with Nowak and Bangham (51), our data support the assumption that virus load and CTL response are linked in a dose-dependent fashion (36, 52).

We propose that the extent of CD8+ T cell expansion leading to primary CTL activity during an acute viral infection is dependent on virus load, i.e., contacts between specific CD8+ T cell and epitope-presenting (infected) cells. In this way, minor epitope-specific CD8+ T cells are activated during WE infection but fail to expand to a detectable level in a primary CTL response. We conclude that important features of CTL populations specific for minor epitopes are their slower development and their longer requirement for stimuli by higher virus load compared with those of CTL specific for major epitopes. By the experiments reported here, we cannot decide whether this feature is a function of the possibly lower precursor frequency of CTL specific for minor epitopes or if expansion of these cells is lower, possibly because of the lower TCR affinity to peptide-MHC complexes of minor epitopes.

However, during days 6 to 8 of ESC infection, minor epitope-specific CTL expand due to the 10-fold higher virus level, leading to an increased contact frequency between virus-infected cells and T cells (see Fig. 3). The concept of negative regulation of minor epitope-specific CTL due to a fast decreasing virus load thereby explains the relationship between virus load (stimulus) and the extent of primary CTL response in acute virus infections.

This concept also explains, why CTL against major and minor epitopes arise independently when expressed on different cells. This observation was made in this study during coinfection of mice with LCM virus WE and ESC and also in the CTL responses to minor epitopes of influenza A virus (40). Obviously, when minor epitopes were expressed independently from major epitopes, the faster, major epitope-specific CTL cannot reduce the number of cells this quickly. When both types of epitopes were expressed on the same cell, i.e., by infection of mice with WE virus, the stimulus for minor specific CTL decreases quickly by fast developing major epitope-specific CTL.

Kinetic differences in virus clearance and in the development of lytic activity against different epitopes were observed by others, describing a shift from major to minor epitopes when the response against major epitopes was abrogated (47, 48, 50). In agreement with our hypothesis, minor epitope-specific CTL develop slower, and virus elimination is prolonged in the MHC class I molecule loss variant C-H-2<sub>dm2</sub> mice during LCM virus infection (17, 53).
According to our data, the classification of an epitope as major or minor reflects the responsiveness of epitope-specific CTL precursors to expand to a given stimulus. This property is determined by the T cell repertoire and the MHC setting. In this way, the context of any epitope to other epitopes in a complex Ag does not influence this classification. In contrast, the terms dominant and subdominant describe the relative strength of CTL responses against the individual epitopes induced by a complex Ag. This could be demonstrated in experiments using F1 hybrid mice. In these mice we compared the relative hierarchy of viral epitopes to those of the parental mouse strains. We found that n118 in BALB/c and the H-2b-restricted epitopes in B6 mice are major epitopes at the same hierarchical level. Development of lytic activity against the minor LCM virus epitope in BALB/c mice was suppressed when CTL against the major H-2b epitopes were present.

Our model of a kinetic control of the CTL response has practical consequences for vaccine development. To prevent the selection of CTL escape variants, CTL should be induced against a sufficient number of major epitopes. When CTL against the major H-2b epitopes were present.

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