JOURNAL OF VIROLOGY, Oct. 2005, p. 12703–12713 Vol. 79, No. 20
0022-538X/05/$08.00+0 doi:10.1128/JVI.79.20.12703–12713.2005
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Rapid Recruitment of Virus-Specific CD8 T Cells Restructures Immunodominance during Protective Secondary Responses

Anne E. Tebo, Michael J. Fuller, Dalia E. Gaddis, Kyoko Kojima, Kunal Rehani, and Allan J. Zajac*

Department of Microbiology, University of Alabama at Birmingham, Birmingham, Alabama 35294-2170

Received 8 April 2005/Accepted 18 July 2005

In this study we investigate the attributes of virus-specific memory CD8 T cells which most effectively control secondary infections. By rechallenging mice that had cleared primary lymphocytic choriomeningitis virus infections, we revealed that the secondary response is remarkably swift. Within 6 h following secondary infection, the production of gamma interferon becomes detectable directly ex vivo. During this protective phase of the secondary response, a very early elaboration of effector activities is preferentially exhibited by T cells specific for the viral NP396 epitope. This wave of activation contains the infection primarily before the initiation of the proliferative phase of the secondary response. Marked expansion is observed, but its magnitude differs depending on the epitope specificity of the responding cells; between 42 and 48 h following infection, ~70% of NP396-specific memory cells are in the S phase of the cell cycle, as assessed by bromodeoxyuridine incorporation studies. Epitope-dependent differences during the proliferative phase of the secondary response were confirmed by adoptive transfer studies with CFSE-labeled T cells. Although NP396-specific T cells typically dominate secondary responses, the broader multiepitope-specific population of antiviral T cells is beneficial for controlling a variant virus with an escape mutation in this epitope. These findings indicate that the induction and maintenance of a focused response contribute to the clearance of secondary infections; however, a more diverse pool of antiviral T cells facilitates long-term immunity to mutable pathogens.

The hallmarks of adaptive immunity, including antigen specificity and memory, are well exemplified by antiviral CD8 T cells. These T cells serve as excellent sentinels by virtue of their ability to survey the peptide derivatives of viral and cellular proteins which are presented in the context of major histocompatibility complex (MHC) class I molecules. During the course of viral infections, a spectrum of peptide epitopes are displayed for inspection by CD8 T cells. Since CD8 T cells express a clonal T-cell receptor, an individual T cell is typically only capable of detecting a specific peptide-MHC complex (24). Consequently, viral infections elicit pools of CD8 T cells which recognize arrays of distinct epitopes. The magnitude and breadth of these discrete responses are not necessarily equal; therefore, a hierarchy of immunodominance can emerge as certain specificities of antiviral T cells can predominate (23, 67). Several reports have shown preferential targeting of particular viral epitopes by CD8 T cells during the acute or chronic phase of persistent infections, including infections with human immunodeficiency virus (HIV), simian immunodeficiency virus (SIV), Epstein-Barr virus, and hepatitis C virus (17–20, 25, 31, 40, 46, 52, 58, 65). Thus, although a variety of responses may be induced, it is plausible that only select specificities of CD8 T cells best contribute to prolonged protective immunity.

Primary acute viral infections drive the development of long-lived pools of memory CD8 T cells (19, 32, 44, 50, 63). In this way, a beneficial memory of past antigenic experiences is established as an increased number of virus-specific T cells are maintained which are tuned to rapidly respond if they reencounter infected cells. Thus, if reexposure to the initial pathogen occurs, a preexisting population of antiviral T cells is available that can quickly elaborate effector functions which remove infected target cells before progeny virus is released. This ability to mount an accelerated recall response is a distinguishing trait of immunological memory (50, 63). Nevertheless, the overall population of memory T cells is heterogenous and comprised of phenotypically and functionally diverse subsets specific for a variety of viral epitopes (23, 64, 67). A key issue for infection control and vaccine development is delineating which components of this memory T-cell pool are most effective at clearing pathogens following secondary exposures.

Lymphocytic choriomeningitis virus (LCMV) infection of mice serves as an ideal system to probe the complexity of memory T-cell subsets. Acute infections of adult mice with this natural mouse pathogen elicit a massive virus-specific CD8 T-cell response (10, 13, 44, 62). This response is required for the resolution of the infection, and in H-2^b mice the overall primary response is directed towards at least 10 epitopes (58, 59). As the primary response is downsized, populations of memory CD8 T cells emerge and are stably maintained over time (19, 32, 44). These memory cells confer resistance to intracranial rechallenge, which is lethal to naive mice, and also more rapidly control peripheral exposures to virulent viral isolates (10, 62). Thus, the LCMV system is a particularly suitable platform for examining the biological importance of memory CD8 T-cell subsets and how they participate in protective recall responses.

For the current study, we utilized the LCMV system to address how the ensemble of virus-specific memory CD8 T

* Corresponding author. Mailing address: Department of Microbiology, University of Alabama at Birmingham, 845 Nineteenth Street South, 446 BBRB, Birmingham, AL 35294-2170. Phone: (205) 975-5644. Fax: (205) 975-5645. E-mail: azajac@uab.edu.

cells participates during the course of secondary viral exposures. Our findings show that reinfection of LCMV-immune mice induces a wave of activation of preexisting memory T cells which is detectable as early as 6 h after challenge. Although the secondary response is rapidly initiated, striking epitope-dependent differences are observed as "first responders" are preferentially recruited. This suggests that the differential maintenance and recruitment of epitope-specific T-cell subsets impact the capacity to optimally control secondary infections.

MATERIALS AND METHODS

Mice and viruses. C57BL/6 (B6) and B6.Cg-*Igha Thy1a Gpi1a*/J (Thy 1.1) mice, both H-2^b, were purchased from The Jackson Laboratory (Bar Harbor, ME). Animals were bred and maintained in accredited facilities at the University of Alabama at Birmingham. For primary infections, 6- to 10-week-old mice were infected by intraperitoneal injection using 2×10^5 PFU of LCMV-Armstrong. For rechallenge studies, immune mice (≥3 months after primary LCMV-Armstrong infection) were administered 2×10^6 PFU of LCMV clone 13 intravenously (i.v.). In certain experiments, naive mice were similarly inoculated to establish primary LCMV clone 13 infections. All viral isolates were originally provided by R. Ahmed (Emory University, Atlanta, GA) and were propagated in BHK-21 cells. The titers of viral stocks and tissue samples were determined by plaque assays using Vero cell monolayers (2).

Cell preparation. Spleen and liver cells were prepared as described previously (36). Briefly, livers and spleens were harvested from mice following perfusion with 10 ml of phosphate-buffered saline (PBS). Spleens were disrupted into single-cell suspensions, and erythrocytes were removed by treatment with 0.83% (wt/vol) NH_4Cl. Suspensions of liver cells were prepared, and liver lymphocytes were collected over a layer of Histopaque-1083 (Sigma-Aldrich, St. Louis, MO). After being washed, all cell preparations were resuspended in RPMI 1640 medium supplemented with 10% fetal calf serum (FCS), 50 μM 2-mercaptoethanol, 100 U/ml penicillin, and 100 μg/ml streptomycin.

MHC class I tetramer preparation. MHC class I tetramers were prepared as previously described (20). Monomers were formed by refolding H-$2D^b$ or H-$2K^b$ heavy chains with antigenic peptides and human β_2-microglobulin. Following purification, tetramers were formed by the stepwise addition of allophycocyanin-conjugated streptavidin (Molecular Probes, Eugene, OR).

MHC class I tetramer staining. Suspensions of spleen and liver cells were treated with anti-CD16/CD32 monoclonal antibody (clone 2.4G2) prior to staining with MHC class I tetramers and anti-CD8 antibody. For costaining with H-$2D^b$ tetramers, the anti-CD8–phycoerythrin (PE) antibody clone 53-6.7 (BD Pharmingen, San Diego, CA) was used; however, the anti-CD8–PE antibody clone CT-CD8a (Caltag, Burlingame, CA) was used in conjunction with H-$2K^b$ tetramers. Fluorescein isothiocyanate (FITC)-conjugated antibodies were included to assess the expression of CD43 (1B11), CD44 (IM7), and CD62L (MEL-14). Cells were stained on ice in PBS, 2% (wt/vol) bovine serum albumin, and 0.2% (wt/vol) NaN_3 (fluorescence-activated cell sorting [FACS] buffer) and subsequently washed three times in FACS buffer and fixed in PBS–2% (wt/vol) paraformaldehyde. Samples were acquired using a FACSCalibur flow cytometer (BD Biosciences, San Jose, CA), and data were analyzed using the computer program Cell Quest.

Ex vivo intracellular cytokine staining. Intracellular cytokine staining was performed as previously described, but without additional peptide stimulation or brefeldin A treatment (14, 20). Briefly, freshly prepared suspensions of spleen and liver cells were costained with MHC class I tetramers and anti-CD8 antibodies as described above. Intracellular staining was performed using anti-gamma interferon (IFN-γ)–FITC antibody (clone XMG1.2; BD Pharmingen, San Diego, CA) in accordance with previously described methodologies (20).

BrdU incorporation. The proliferation of antigen-specific CD8 T cells in vivo was monitored by measuring bromodeoxyuridine (BrdU; Sigma, St. Louis, MO) incorporation. For short labeling experiments, 0.8 mg BrdU in PBS was administered to mice by intraperitoneal injection 6 h before sacrifice. For long labeling experiments, BrdU (0.8 mg/ml) was given in daily changes of drinking water for a period of 6 days. To detect BrdU incorporation, freshly explanted splenocytes were stained with MHC class I tetramers and anti-CD8–PE antibodies. Stained samples were then fixed and permeabilized by overnight treatment at 4°C with 1% (wt/vol) paraformaldehyde and 0.05% Igepal (Sigma, St. Louis, MO) in PBS. The cells were then washed twice with PBS and incubated, with periodic mixing, for 30 min at 37°C in PBS containing 4.2 mM $MgCl_2$ and 50-Kunitz units/ml DNase I, pH 5 (Sigma, St. Louis, MO). Cells were subsequently stained with a 1:20 dilution of anti-BrdU–FITC antibody (clone 3D4; BD Pharmingen San Diego, CA) prepared in PBS containing 5% FCS, 2% mouse serum, and 0.5% Igepal. After 45 min on ice, samples were washed twice and finally resuspended in PBS–1% (wt/vol) paraformaldehyde. Samples were analyzed by flow cytometry as described above.

CFSE labeling and adoptive transfers. Splenocytes were prepared from immune Thy1.1 mice >3 months following primary infection with LCMV-Armstrong. CD8 T cells were enriched by positive selection with magnetic beads (Miltenyi, Auburn, CA) and then washed with PBS. Isolated cells were at least 87% $CD8^+$. Cells (10^7 cells/ml) were then labeled with 4 μM 5 (and 6-)-carboxyfluorescein diacetate, succinimidyl ester (CFSE) (Molecular Probes, Eugene, OR) for 8 min at 25°C. Labeling was quenched by the addition of FCS, and cells were washed twice with PBS. CFSE-labeled CD8 T cells (~5×10^6) from LCMV-immune Thy1.1 mice were then transferred by i.v. injection into either naive or LCMV-immune B6 mice which had been primed with LCMV-Armstrong at least 3 months previously. The following day, recipient mice were either left untreated or infected with 2×10^6 PFU LCMV clone 13 i.v. On days 1, 2, and 4 following infection, recipient mice were sacrificed, and splenocytes were prepared as described above. Cells were costained with anti-Thy1.1–PE antibody (clone OX-7; BD Pharmingen) and MHC class I tetramers. Samples were analyzed by flow cytometry, and the diminution in green fluorescence was determined.

Isolation of LCMV variant F403L. Viral isolates were cloned from serum samples obtained from C57BL/6-$Cd4^{tm1Mak}$ ($CD4^{-/-}$) mice on day 287 following LCMV clone 13 infection. Following three rounds of plaque purification on Vero cell monolayers, viral isolates were grown in BHK-21 cells for 2 days (2). Total RNAs were extracted from cell lysates and used as templates for the generation of viral GP and NP cDNAs by reverse transcription-PCR. The primers used to reverse transcribe and amplify the GP region were 5′-AAGTGGTTCCTCATC AGTAGTTG-3′ and 5′-TGTTTGAGGCTCTGCCTCACATC-3′. For the NP region, the following primers were used: 5′-CTTAACAACACAGCAACTAGA CC-3′ and 5′-TCCTATTCTGTGAGTCCAGAAGC-3′. PCR products were purified following gel electrophoresis and then sequenced. A viral variant was identified, designated F403L, which possessed a phenylalanine-to-leucine amino acid coding change at residue 403 of the viral NP, within the H-$2D^b$-restricted NP396 epitope. The amino acid sequences of all other known H-2^b epitopes remained unaltered; however, by comparison with the parental virus, a noncoding U-to-C mutation was identified at nucleotide 299 of the viral GP gene and a leucine-to-phenylalanine amino acid change was present at GP residue 252.

RESULTS

Accelerated viral clearance in immune mice. Primary acute infection with the Armstrong strain of LCMV induces a potent antiviral CD8 T-cell response which eradicates the infection (reviewed in references 10 and 62). To confirm that immunological memory was established following this acute infection, immune mice which had been infected with LCMV-Armstrong >3 months previously were rechallenged with the virulent viral isolate clone 13. Although replicating virus was initially detectable following rechallenge, the secondary infection was swiftly controlled, and by day 3 the viral titers were below the limits of detection in all samples checked (Fig. 1). In contrast, viral titers remained high 4 days following primary infection of naive mice with a similar dose of LCMV clone 13 (Fig. 1), and eventually this infection was slowly controlled over a period of 2 to 3 months (19, 65; data not shown). Overall, these data are consistent with the concept that rather than conferring sterilizing immunity, memory CD8 T cells rapidly removed virus-infected cells, thus reducing viral dissemination and facilitating clearance. We utilized this rechallenge approach to probe whether discrete subsets of antiviral memory CD8 T cells differentially contribute to the anamnestic response following viral rechallenge.

Rapid recruitment of memory CD8 T cells is revealed by ex vivo IFN-γ production. Acute LCMV infection establishes pools of memory CD8 T cells which recognize various distinct

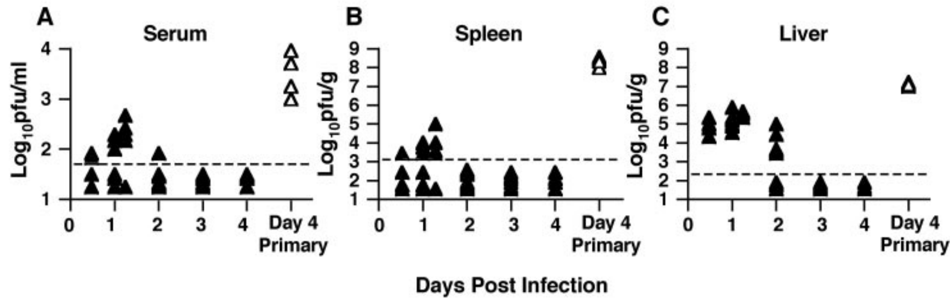

FIG. 1. Accelerated viral clearance following secondary infection. Viral titers were quantified in sera (A), spleens (B), and livers (C) by plaque assays at various time points following rechallenge of immune mice with the clone 13 strain of LCMV (▲). As a control, naive mice were similarly infected with LCMV clone 13, and titers were assessed 4 days following primary infection (△). Titers of four to eight individual mice analyzed at each time point are shown, and dashed lines indicate limits of detection.

viral epitopes (44, 58, 59). To address whether the functional recruitment of these antiviral T cells differed depending on their epitope specificity, direct ex vivo intracellular analysis of IFN-γ was performed without additional in vitro peptide stimulation. As expected, resting virus-specific memory CD8 T cells in the spleens and livers of LCMV-immune mice did not express detectable levels of IFN-γ (Fig. 2). In contrast, IFN-γ production by select specificities of antiviral T cells was detectable as early as 6 h following secondary challenge. The proportion of IFN-γ^+ MHC tetramer$^+$ CD8 T cells was maximal by 12 and 24 h post-secondary infection in spleens and livers, respectively. Thus, a transient rise in viral titers following reinfection was accompanied by the activation of IFN-γ production by antiviral CD8 T cells. This was rapidly initiated and detectable without additional stimulation but was short-lived, as IFN-γ production declined markedly by 48 h postinfection and was below the limits of detection by 72 h. Although all specificities of antiviral CD8 T cells produced IFN-γ to some

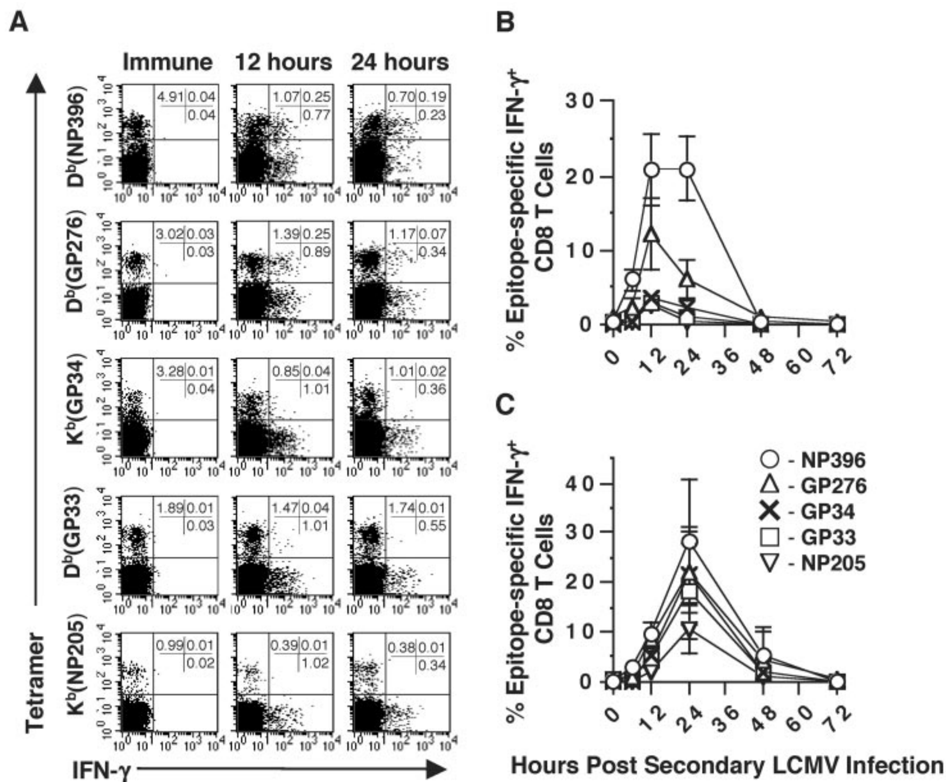

FIG. 2. Rapid but differential production of IFN-γ by virus-specific CD8 T cells during recall responses. (A) Representative flow cytometry plots show MHC class I tetramer and IFN-γ staining profiles of gated splenic CD8 T cells from immune mice and from mice 12 and 24 h following rechallenge. The values represent the percentages of CD8 T cells present in each respective quadrant. Line graphs show the mean percentages ± standard deviations (SD) of epitope-specific IFN-γ^+ T cells in the spleen (B) and liver (C) at various time points following rechallenge. Three to eight mice were analyzed at each time point.

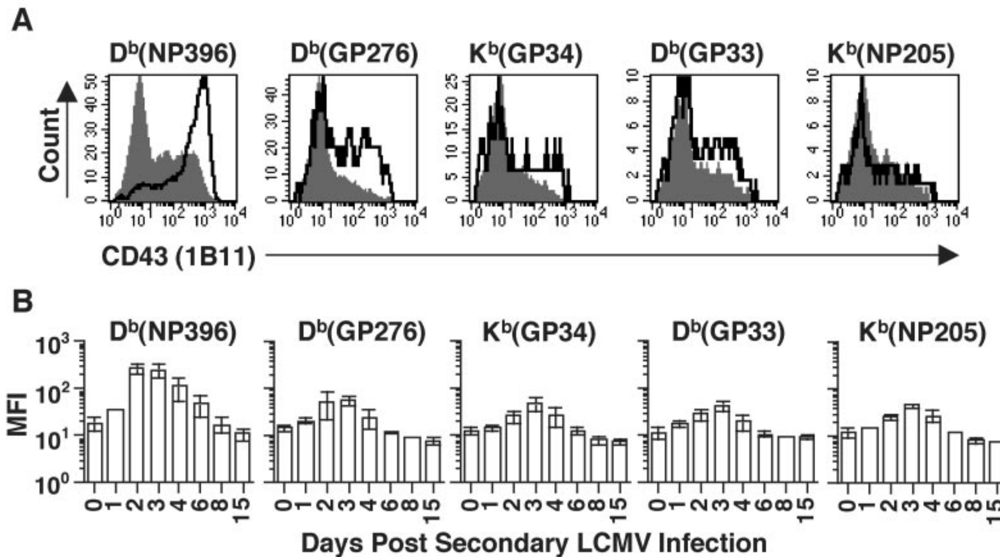

FIG. 3. Disparities in virus-specific memory CD8 T-cell activation. Changes in the expression of CD43 (1B11) on epitope-specific CD8 T cells were examined at various time points following rechallenge of LCMV-immune mice. (A) Histogram plots show representative CD43 staining profiles of splenic epitope-specific CD8 T cells from either immune mice (gray shading) or mice at day 2 post-secondary infection (black line). (B) Mean fluorescence intensities (MFI) ± SD of CD43 expression on epitope-specific CD8 T cells on various days following viral rechallenge. Three to eight mice were analyzed at each time point.

extent, a striking differential activation was observed. The activation of NP396-specific CD8 T cells was clearly favored, and by 12 h following rechallenge, 21% ± 5% of these cells were IFN-γ^+ in the spleen. Shifts in immunodominance were also apparent, as GP276-specific CD8 T cells formed the next most prominent response, whereas GP33- and NP205-specific pools were less activated.

Selective activation of epitope-specific memory CD8 T cells. We next addressed whether the observed differences in functional activation were accompanied by discrete changes in surface marker expression on the responding CD8 T cells (Fig. 3 and 4). Resting memory T cells express low levels of the 1B11 activation-associated isoform of CD43, but expression of this glycosylated form is upregulated following reexposure to antigen (30). Conversely, resting memory CD8 T cells typically express high levels of the adhesion molecule CD62L, while its expression is decreased on recently activated cells (54).

By 2 days post-secondary infection, 79% ± 5%, 36% ± 11%, 26% ± 5%, 24% ± 6%, and 19% ± 4%, of NP396-, GP276-, GP34-, GP33-, and NP205-specific CD8 T cells were $CD43^{hi}$, respectively (Fig. 3). This initial increase in CD43 expression differed depending on the epitope specificity of the antiviral CD8 T cells, and the extent of activation was typically consistent with the patterns of IFN-γ expression shown in Fig. 2; however, maximal expression of CD43 occurred after the peak of ex vivo IFN-γ production. Although all specificities of antiviral CD8 T cells checked had increased CD43 expression, this upregulation was most marked for NP396-specific CD8 T cells (Fig. 3). Nevertheless, expression returned to basal levels by about day 8 following secondary infection.

Shifts in CD62L expression were clearly discernible by 3 days post-secondary infection (Fig. 4). The proportion of $CD62L^{lo}$ cells differed depending on the epitope specificity of the responding cells, and the hierarchy was as follows: NP396 > GP276 > GP34 > GP33 > NP205. Again, this ordered but unequal recruitment was consistent with the observed ex vivo production of IFN-γ as well as the upregulation of CD43. The $CD62L^{lo}$ cells that emerged following viral rechallenge did not rapidly revert to a $CD62L^{hi}$ state, suggesting that the restimulation of memory T cells may cause more long-lived phenotypic imprinting (Fig. 4B). Taken together, the changes in expression of both CD43 and CD62L reflected preferential recruitment of the NP396-specific CD8 T cells following secondary infection.

Unequal proliferation of virus-specific CD8 T cells during recall responses. The epitope-dependent differences in ex vivo IFN-γ production and changes in surface marker expression during the protective phase of the secondary immune response prompted us to examine whether the preferential activation of select specificities of antiviral CD8 T cells was paralleled by differences in antigen-driven proliferation. To address this, BrdU was administered at various time points following viral rechallenge, and mice were sacrificed 6 h later. By staining with MHC class I tetramers and for BrdU incorporation, the proportion of epitope-specific CD8 T cells which were in S phase of the cell cycle during this 6-h labeling period was monitored. As expected, no marked turnover of resting memory CD8 T cells was observed in LCMV-immune mice (Fig. 5A, top row). Similar basal levels of BrdU incorporation were observed within the first 6 h following rechallenge; however, between 42 and 48 h following rechallenge, remarkable levels of BrdU incorporation were observed. Within this 6-h period, ~70% and ~50% of NP396- and GP276-specific CD8 T cells, respectively, became $BrdU^+$ (Fig. 5B). Lower levels of BrdU incorporation were observed for other specificities of antiviral T cells. BrdU incorporation continued to be observed during the third day (66 to 72 h) following secondary infection but subsided by day 5 and continued to return to basal levels (Fig. 5A

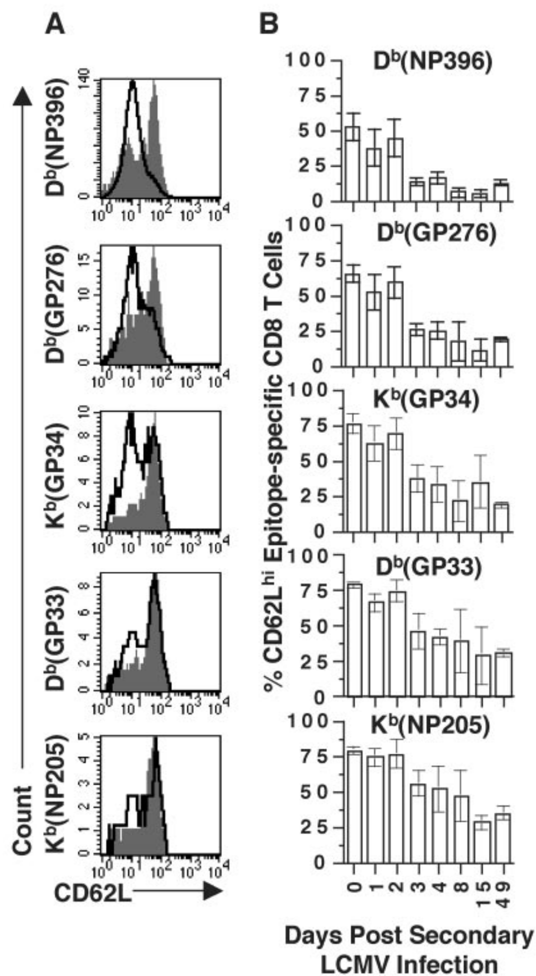

FIG. 4. Downregulation of CD62L during recall responses. Changes in the expression of CD62L on epitope-specific CD8 T cells were examined at various time points following rechallenge of LCMV-immune mice. (A) Histogram plots show representative CD62L staining profiles of splenic epitope-specific CD8 T cells from either immune mice (gray shading) or mice at day 3 post-secondary infection (black line). (B) Percentages ± SD of CD62L^{hi} epitope-specific CD8 T cells at various days following viral rechallenge. Three to eight mice were analyzed at each time point.

and B). The rather striking differential proliferation revealed by 6-h BrdU treatments was consistent with the ex vivo IFN-γ and surface marker analyses, but this expansion phase was primarily initiated after the secondary infection was controlled. Notably, such proliferative differences were not discernible if BrdU was continuously provided in the drinking water for 6 days following rechallenge, as under these conditions >90% of all antiviral CD8 T cells were BrdU$^+$ at the time of analysis (data not shown).

To further investigate the proliferative recruitment of virus-specific memory cells, CFSE-labeled CD8 T cells from immune Thy1.1 mice were transferred into naive or LCMV-immune Thy1.2 mice, and the recipients were subsequently challenged with 2×10^6 PFU of LCMV clone 13. In this way, the proliferation of memory T cells could be tracked in an environment where the infection becomes widely disseminated (naive mice) or under conditions of a limiting antigen load, which results from the rapid clearance of virus in immune recipients (Fig. 1). At 1, 2, and 4 days postinfection, the proportions of NP396-, GP276-, and GP33-specific donor cells recovered from the spleen were examined. At day 1 postinfection, no pronounced proliferation of virus-specific donor cells was discernible in either naive (Fig. 6A) or immune (Fig. 6B) recipients. In naive mice, few donor cells were recoverable by 2 days following infection, and the staining patterns were reproducibly diffuse. Nevertheless, the more disseminated infection in these naive hosts resulted in extensive recruitment and proliferation, since by 4 days postinfection >95% of the epitope-specific donor cells had divided at least seven times (Fig. 6A).

The rapid control of the secondary LCMV infection in immune mice was associated with an unequal recruitment of memory CD8 T cells which depended on the viral epitope recognized by the responding cells. By 2 days postinfection, NP396-specific cells had undergone up to five divisions in immune recipients, but GP276- and GP33-specific donor cells participated less in the recall response. Calculations of the recruitment of CFSE-labeled cells (28) revealed that 67% ± 5%, 29% ± 6%, and 14% ± 3% of NP396-, GP276-, and GP33-specific donor cells, respectively, had responded to the secondary infection at this time point. This stratification was consistent with the BrdU incorporation data (Fig. 5). The discordant recruitment of epitope-specific CD8 T cells in immune hosts continued to be observed by 4 days following infection, since unlike naive recipients, not all of the donor cells had undergone seven or more divisions. To confirm that the division of donor cells was driven by the viral infection, CFSE-labeled cells were similarly transferred into naive or immune recipients that were not subsequently challenged with LCMV. The donor cells remained undivided in these virus-free recipients (Fig. 6A and B, bottom panels).

Taken together, the results from naive mice indicate that all subsets of epitope-specific memory CD8 T cells retain the capacity to mount anamnestic responses, especially if the antigen dose is not limiting. The more limited viral replication that ensues following secondary infection is, however, associated with the rapid early recruitment of select specificities of antiviral CD8 T cells. This both promotes viral control and structures the hierarchy of the virus-specific T-cell pool.

Kinetics of virus-specific recall responses. CD8 T-cell responses in the spleens and livers were enumerated at various time points following secondary viral challenge to determine whether the disparities in proliferative recruitment translated into disproportionate changes in the absolute numbers of epitope-specific cells (Fig. 7). Within the first 24 h following rechallenge, the numbers of epitope-specific CD8 T cells declined. This decrease coincided with the elevation of viral titers (Fig. 1) and the production of IFN-γ (Fig. 2). By day 2, the responding cells had begun to enter the cell cycle (Fig. 5 and 6). The resulting expansion phase occurred as the infection was mostly contained (Fig. 1) and following the cessation of IFN-γ production (Fig. 2) but was associated with changes in surface marker expression (Fig. 3 and 4). The overall kinetic profile of the recall response (Fig. 7) was consistent with the differential proliferative recruitment of epitope-specific CD8 T cells, revealed by BrdU incorporation (Fig. 5) and CFSE labeling (Fig. 6). NP396-specific T cells predominated, followed by those

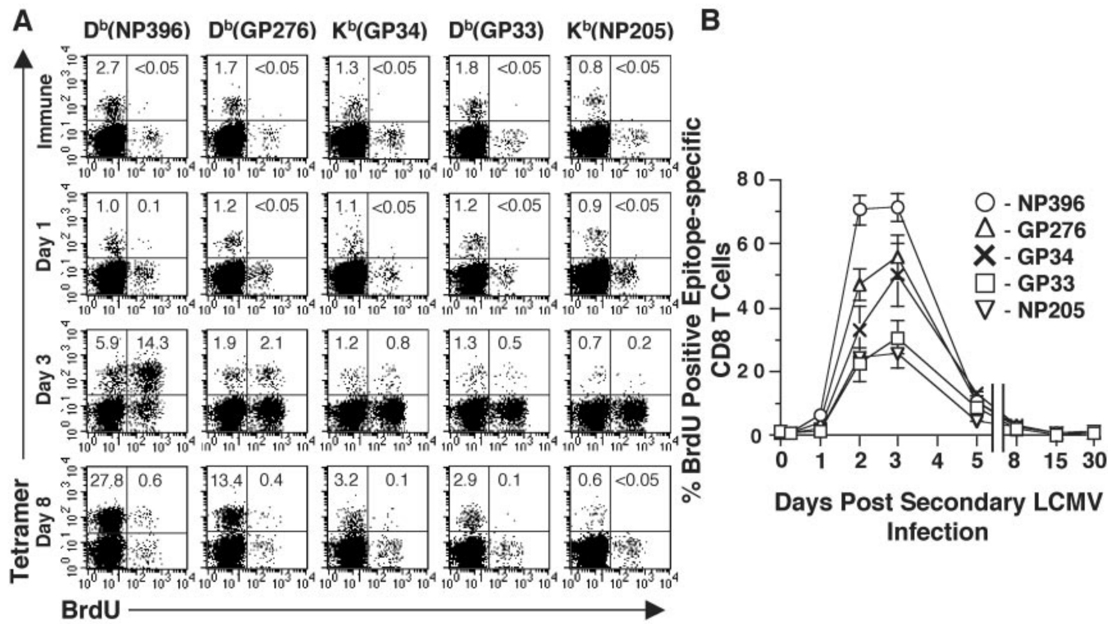

FIG. 5. Proliferation of select specificities of antiviral T cells during secondary responses. (A) Representative flow cytometry plots show tetramer and anti-BrdU straining profiles of gated CD8 T cells from either LCMV-immune mice or rechallenged immune mice on days 1, 3, and 8 following secondary LCMV infection. In each case, BrdU was administered 6 h prior to analysis. The values given in the upper left and right quadrants represent the percentages of $CD8^+$ T cells that are either $tetramer^+$ $BrdU^-$ or $tetramer^+$ $BrdU^+$, respectively. (B) Kinetics of BrdU incorporation by antigen-specific CD8 T cells following secondary infection. Mean values ± SD of two to seven mice analyzed at each time point are plotted.

specific for the GP276 epitope. In comparison with immune mice, the mean increase in the number of splenic epitope-specific CD8 T cells 6 days following secondary infection was 59.3-, 41.6-, 23.1-, 10.9-, and 3.3-fold for NP396-, GP276-, GP34-, GP33-, and NP205-specific cells, respectively; similarly, in the liver, the expansion of these epitope-specific cells was 77.2-, 64.0-, 40.8-, 48.9-, and 16.0-fold. Whereas during primary LCMV infection a dramatic contraction of epitope-specific CD8 T cells occurs following the peak of the effector phase of the response, the secondary response was more slowly downsized, as the numbers of epitope-specific CD8 T cells declined gradually overtime. This is consistent with studies showing that memory CD8 T cells are more resistant than primary effectors to cell death (6, 26). Overall, these data indicate that the early differential activation and proliferation of CD8 T cells during recall responses impact the hierarchical structure and long-term abundance of the antiviral T-cell pool.

Alteration of the immunodominant epitope impacts recall responses. Given the striking early recruitment of NP396-specific CD8 T cells, we evaluated whether rechallenge of LCMV-immune mice with a variant virus encoding a mutation within this immunodominant epitope resulted in compensatory changes in the recall responses. As part of ongoing studies, we isolated a variant LCMV clone 13 virus, designated F403L, that differs from wild-type LCMV clone 13 due to a point mutation resulting in a phenylalanine-to-leucine amino acid change at residue 403 of the viral nucleoprotein. This substitution is at position 8 of the NP396 antigenic peptide. Previous studies of viral variants in the LCMV-Armstrong background have shown that this altered epitope is capable of binding to $H\text{-}2D^b$ but exhibits dramatically reduced immunogenicity (41).

LCMV-immune mice were rechallenged with either LCMV clone 13 or the F403L variant, and the hierarchy of the secondary T-cell response was analyzed after 5 days (Fig. 8). As expected, the immunodominance of the recall response in both spleens (Fig. 8A) and livers (Fig. 8B) following secondary infection with LCMV clone 13 was ordered as follows: NP396 $>$ GP276 $>$ GP34 $\geq$ GP33 $>$ NP205. Following rechallenge with the F403L variant, the burst size of the secondary NP396-specific response was markedly reduced. A shift in immunodominance was observed, as GP276-specific cells now predominated, followed by GP34-specific cells. Nevertheless, the magnitudes of these individual epitope-specific responses were lower and not as striking as the usually prominent NP396-specific population elicited by rechallenge with wild-type virus. Notably, viral clearance was not compromised by the F403L mutation, as viral titers were below the limits of detection in the serum and lungs 5 days following rechallenge with either the wild-type or F403L variant virus (data not shown). Thus, an amino acid mutation within the usually dominant NP396 epitope can be immunologically tolerated by a broad overall secondary virus-specific CD8 T-cell response.

DISCUSSION

Since CD8 T cells eliminate infected cells rather than neutralizing circulating virus, it is critical that they quickly elaborate effector functions in order to contain the secondary infec-

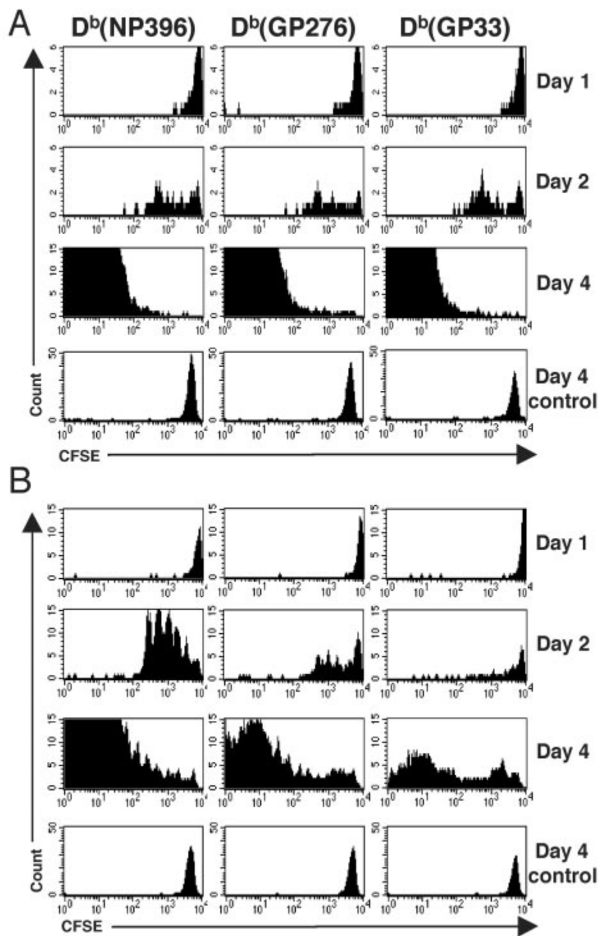

FIG. 6. Rapid control of secondary LCMV infection limits memory CD8 T-cell recruitment. CFSE-labeled CD8 T cells from immune Thy1.1 donor mice (5×10^6) were adoptively transferred into either Thy1.2 naive (A) or immune (B) recipients. One day later, mice were infected with 2×10^6 PFU of LCMV clone 13 or left as unchallenged controls. The division of donor cells recovered from the spleens of recipient mice was determined by monitoring the diminution of CFSE fluorescence on days 1, 2, and 4 following infection. Control mice were analyzed in parallel with the day 4 postinfection group. Each panel shows the intensity of green fluorescence on gated Thy1.1 donor cells, which stain positively with the indicated MHC class I tetramer. Representative data from one of two similar experiments are shown.

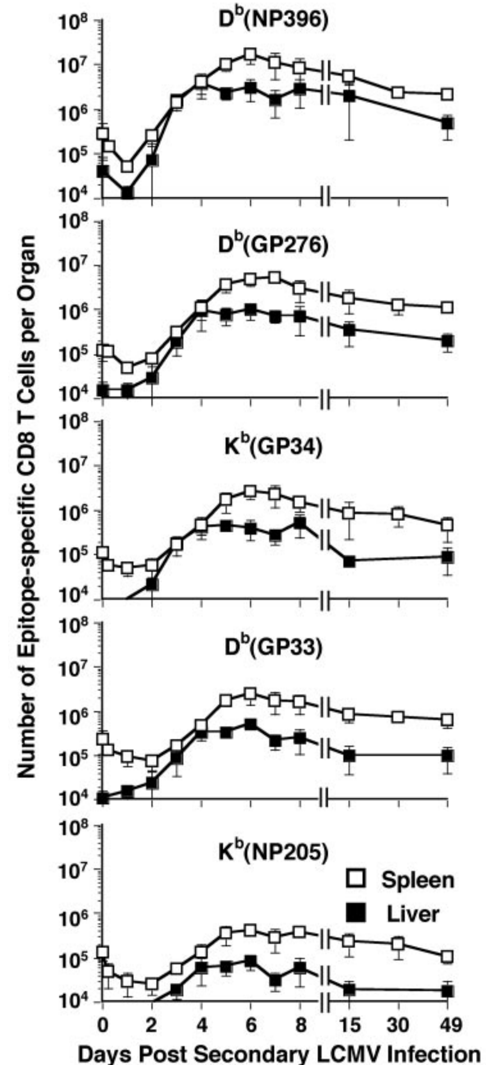

FIG. 7. Kinetics of epitope-specific secondary CD8 T-cell responses. Virus-specific CD8 T-cell responses in spleens (□) and livers (■) were enumerated by MHC class I tetramer costaining at various time points following rechallenge of immune mice with LCMV clone 13. Mean values ± SD are plotted for four to nine mice analyzed at each time point.

tion. Whereas the activation of naive cells causes the acquisition of effector properties following a proliferation and differentiation program, which can take days as the infection progresses in vivo, a distinguishing feature of memory CD8 T cells is that they can lyse target cells and secrete IFN-γ without undergoing additional cell-division-associated differentiation (15, 35, 39, 60, 61). Thus, although resting memory CD8 T cells do not constitutively exert their antiviral activities, they are poised to act expeditiously upon reengaging cells presenting cognate antigen (51). This is well illustrated by the production of IFN-γ, which becomes detectable directly ex vivo as early as 6 h following reinfection, and the preferential recruitment of NP396-specific T cells is apparent even at this early stage (Fig. 2). The triggering of effector activities occurs prior to the subsequent expansion phase of the secondary response, which is most pronounced only after the infection is largely contained. Consequently, "first responders" are likely to play a more significant role in the early containment of the infection by rapidly eliminating infected cells, thus limiting viral spread as well as preventing the recruitment of other epitope-specific T-cell subsets.

The pronounced participation of NP396-specific CD8 T cells appears to be a general feature of the secondary response to LCMV, as this trend is observed following reinfection with either LCMV clone 13 (this report) or LCMV-Armstrong (A. E. Tebo and A. J. Zajac, unpublished observations). The hierarchy of the pool of virus-specific T cells is shaped by a multifactorial process; however, we favor the hypothesis that the temporal order of viral protein synthesis can act as a

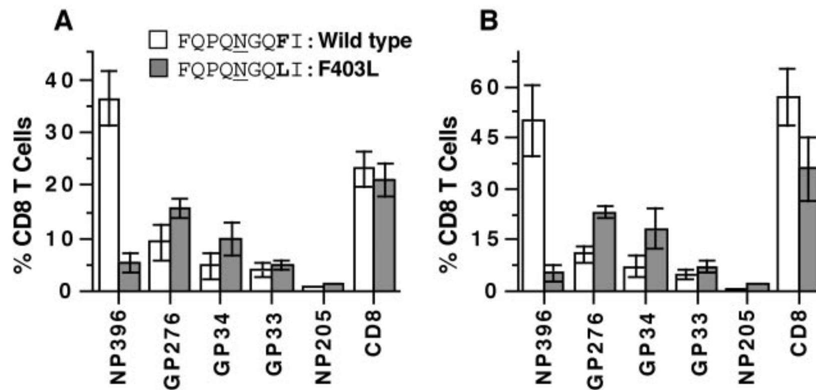

FIG. 8. Broad compensatory secondary responses following rechallenge with an NP396 variant virus. LCMV-immune mice were rechallenged with 2×10^6 PFU of either LCMV clone 13 (white bars) or the F403L variant virus (gray bars), and epitope-specific CD8 T-cell responses were analyzed by tetramer costaining 5 days later. Plots show the mean percentages ± SD of epitope-specific CD8 T cells as well as the overall percentages of CD8 cells recovered from the spleens (A) and livers (B) of seven or eight rechallenged mice analyzed from each group. The primary amino acid sequences of the wild-type and F403L epitopes are indicated, with the mutation highlighted in bold and the N anchor residue underlined. Cell recoveries were $1.34 \times 10^8 \pm 2.5 \times 10^7$ and $8.64 \times 10^6 \pm 4.0 \times 10^6$ from the spleens and livers, respectively, following LCMV clone 13 rechallenge and $7.57 \times 10^7 \pm 2.4 \times 10^7$ and $6.18 \times 10^6 \pm 1.6 \times 10^6$ from the spleens and livers, respectively, following rechallenge with the F403L variant.

principle cause for the structuring of immunodominance following secondary infections (23, 47, 48, 58, 67). In infected cells, LCMV NP mRNAs have been shown to accumulate 9 to 12 h before GP mRNAs, as the transcription of GP mRNA is delayed until complementary viral genomes have been synthesized (4, 21). This is consistent with studies of viral proteins showing that NP is detectable in infected cells prior to the accumulation of GP (9). Moreover, studies by van den Broek and coworkers have further shown that NP-derived epitopes are presented for inspection by T cells 5 to 8 h before GP-derived epitopes (47). Consequently, following secondary infections, preexisting memory T cells are likely to first encounter NP epitopes. Nevertheless, if the infected cell is not quickly eliminated and viral replication is allowed to proceed, then the balance of epitope presentation may shift. Estimations of the abundance of epitopes upon MC57 fibroblasts 48 h following infection indicate that the processed epitopes are presented by H-2D^b molecules in the following order: GP33 $\gg$ NP396 $>$ GP276 (22). Thus, the timing of viral protein synthesis and antigen presentation, rather than the absolute abundance of epitopes eventually presented, may be a more critical determinant of the very rapid recruitment of memory cells driving early activation, and most importantly, bringing about efficient viral clearance. The potential utility of targeting early viral proteins has been highlighted by studies of HIV and SIV (27, 56). Frequent responses to the accessory proteins Tat and Rev, which are produced early following infection, have been documented (1). These responses can exert selective pressure for the emergence of viral variants during the acute phase of the infection, but the induction of anti-Tat and anti-Rev responses by candidate vaccines has also been shown to more effective at controlling SIV challenge than vaccines which elicit responses to late viral protein products (3, 45, 53).

The capacity of the immune response to quickly identify infected cells by targeting early viral proteins can clearly contribute to the successful control of secondary infections as well as impact the subsequent hierarchical structure of the response. Nevertheless, immunodominance is a complex in vivo phenomenon which is shaped by many parameters, and the timing of viral protein synthesis cannot solely account for the observed differences that arise following secondary challenge. Notably, both the NP396 and NP205 epitopes are derived from viral NP, but whereas NP396-specific CD8 T cells are vigorously recruited during the recall response, NP205-specific CD8 T cells remain subdominant. The launch of GP276-specific T cells into second place, above the GP33- and GP34-specific responses, following viral rechallenge also cannot be accounted for by the timing of NP verses GP biosynthesis. Each GP molecule can theoretically give rise to one GP276 epitope but can only yield either a K^b GP34 epitope or a D^b GP33 epitope because of sequence overlap (33). Thus, it is tempting to speculate that the abundance of epitopes generated influences the order of the subdominant secondary responses; however, this is not consistent with peptide elution studies, which indicate that more GP33 than GP276 epitopes are presented by 48 h following infection of fibroblasts (22).

The characteristics of the type of cell which presents viral antigens for interrogation by memory T cells may influence the secondary immune response. In vitro studies comparing fibroblasts and dendritic cell lines 3 to 4 days following infection suggest that whereas dendritic cells promote GP33 and NP396 responses, fibroblasts tend to drive GP276-specific responses (12). Investigations with influenza virus further support the notion that the cell type can impact immunodominance, and studies of secondary *Listeria monocytogenes* infections have shown that the destruction of professional antigen-presenting cells impedes the expansion of nonclassical class I (H2-M3) restricted T cells (8, 16, 29). Therefore, not only may differential antigen presentation mold the resulting pattern of secondary immune responses, but also certain specificities of T cells are likely to be more effective at removing particular types of infected cells in vivo.

The pattern of secondary immune responses may also be influenced by intrinsic differences between epitope-specific

pools of CD8 T cells. The functional quality of memory T cells may be imprinted during the primary response by factors such as the timing of activation, abundance of epitopes, stability of interactions, type of cell infected, and duration of antigen presentation. There are indications that NP396-specific CD8 T cells take somewhat longer to downregulate CD43 (1B11) (Fig. 3) and transition into a resting CD62L^{hi} state (Fig. 4). This may indicate that these cells are pretriggered to respond to secondary stimulation and are more capable of infection control. However, intrinsic differences may be overcome by more aggressive infections which recruit additional preexisting pools of epitope-specific memory cells (Fig. 6).

The production of IFN-γ has been shown to shape immunodominance, and several reports have documented that the ability to quickly produce and perceive IFN-γ may promote CD8 T-cell activation (7, 42, 49, 66). Thus, it is plausible that the early production of IFN-γ, which is most pronounced for NP396- and GP276-specific CD8 T cells following rechallenge (Fig. 2), serves to amplify these responses and configures the hierarchy of the secondary response.

The kinetic profile of the responses reveals an appreciable drop in the numbers of detectable splenic virus-specific CD8 T cells within the first day following rechallenge. This occurs concurrently with the elaboration of IFN-γ production and as the response acts to contain the infection. The underlying mechanisms responsible for this apparent loss of T cells are not yet defined but may include trafficking of virus-specific T cells to tertiary tissues so that they are available, if required, to clear virus-infected cells, a downmodulation of T-cell receptor expression due to recent in vivo activation, and apoptosis of T cells early following infection (5, 11, 34, 38, 55, 61).

Changes in immunodominance have been documented not only during secondary immune responses but also during the course of primary infections which are only slowly or never controlled (17–20, 25, 31, 40, 43, 46, 52, 58, 65). In the case of LCMV infection, the NP396-specific response, which is so striking during the successful clearance of secondary infections, is lost during the course of primary LCMV clone 13 infection. Thus, one of the most effective specificities of CD8 T cells for controlling the infection is not maintained in the persistently infected host (19, 47, 58, 65). The kinetics of viral protein synthesis may account for both the marked secondary expansion following rechallenge and the deletion of NP396-specific T cells in the chronically infected host. Whereas early activation can result in the expansion of preexisting memory cells, a more rapid and sustained activation of CD8 T cells during primary infections may drive them to deletion. In the case of HIV infection, cytotoxic T lymphocyte (CTL) responses directed to the early viral proteins Tat and Rev are lost as the infection progresses (1, 57). These shifting patterns of T-cell responses have implications for vaccine design, as epitopes ascribed as immunodominant in chronically infected hosts may not represent the specificities of cells which are most effective at initially controlling the infection.

Although only select specificities of antiviral T cells may play a principle role in controlling secondary infections, flexibility in controlling viral variants with mutations in immunodominant epitopes can be provided by a broad, multiepitope response. We have demonstrated this concept by a rechallenge of immune mice with the LCMV clone 13 variant F403L (Fig. 8). Despite a markedly reduced NP396 response, other specificities of memory CD8 T cells become activated, and the secondary infection is controlled in immune hosts. Polyspecific responses may also be advantageous for counteracting original antigenic sin (37). In these instances, reactivation with variant epitopes may promote responses to the original sequence rather than to the altered peptide, but if other distinct epitopes remain unchanged, then T cells reactive against them can function to control the infection.

Collectively, the findings of this report highlight the benefits of shaping the configuration of the memory T-cell pool so that it is capable of very rapidly clearing viral reexposures. This is accomplished by ensuring that responses against early viral gene products are adequately represented as well as maintaining an overall response which is sufficiently diverse to contain viral variants.

ACKNOWLEDGMENTS

We are grateful to Aaruni Khanolkar, Laurie Harrington, Jason Grayson, and Rodney King for their help and advice. We also thank members of the Wertz laboratory, especially Edward Hinzman, for assistance with viral sequencing.

This work was supported by National Institutes of Health grant AI49360 (to A.J.Z.). M.J.F. and D.E.G. were supported in part by training grants AI07150 and AI07051, respectively.

REFERENCES

1. **Addo, M. M., M. Altfeld, E. S. Rosenberg, R. L. Eldridge, M. N. Philips, K. Habeeb, A. Khatri, C. Brander, G. K. Robbins, G. P. Mazzara, P. J. Goulder, and B. D. Walker.** 2001. The HIV-1 regulatory proteins Tat and Rev are frequently targeted by cytotoxic T lymphocytes derived from HIV-1-infected individuals. Proc. Natl. Acad. Sci. USA **98:**1781–1786.
2. **Ahmed, R., A. Salmi, L. D. Butler, J. M. Chiller, and M. B. Oldstone.** 1984. Selection of genetic variants of lymphocytic choriomeningitis virus in spleens of persistently infected mice. Role in suppression of cytotoxic T lymphocyte response and viral persistence. J. Exp. Med. **160:**521–540.
3. **Allen, T. M., D. H. O'Connor, P. Jing, J. L. Dzuris, B. R. Mothe, T. U. Vogel, E. Dunphy, M. E. Liebl, C. Emerson, N. Wilson, K. J. Kunstman, X. Wang, D. B. Allison, A. L. Hughes, R. C. Desrosiers, J. D. Altman, S. M. Wolinsky, A. Sette, and D. I. Watkins.** 2000. Tat-specific cytotoxic T lymphocytes select for SIV escape variants during resolution of primary viraemia. Nature **407:** 386–390.
4. **Auperin, D. D., V. Romanowski, M. Galinski, and D. H. Bishop.** 1984. Sequencing studies of pichinde arenavirus S RNA indicate a novel coding strategy, an ambisense viral S RNA. J. Virol. **52:**897–904.
5. **Bachmann, M. F., A. Oxenius, D. E. Speiser, S. Mariathasan, H. Hengartner, R. M. Zinkernagel, and P. S. Ohashi.** 1997. Peptide-induced T cell receptor down-regulation on naive T cells predicts agonist/partial agonist properties and strictly correlates with T cell activation. Eur. J. Immunol. **27:**2195–2203.
6. **Badovinac, V. P., B. B. Porter, and J. T. Harty.** 2002. Programmed contraction of CD8(+) T cells after infection. Nat. Immunol. **3:**619–626.
7. **Badovinac, V. P., A. R. Tvinnereim, and J. T. Harty.** 2000. Regulation of antigen-specific CD8+ T cell homeostasis by perforin and interferon-gamma. Science **290:**1354–1358.
8. **Belz, G. T., W. Xie, J. D. Altman, and P. C. Doherty.** 2000. A previously unrecognized H-2D(b)-restricted peptide prominent in the primary influenza A virus-specific CD8^{+} T-cell response is much less apparent following secondary challenge. J. Virol. **74:**3486–3493.
9. **Buchmeier, M. J., J. H. Elder, and M. B. Oldstone.** 1978. Protein structure of lymphocytic choriomeningitis virus: identification of the virus structural and cell associated polypeptides. Virology **89:**133–145.
10. **Buchmeier, M. J., and A. J. Zajac.** 1999. Lymphocytic choriomeningitis virus, p. 575–605. *In* R. Ahmed and I. S. Y. Chen (ed.), Persistent viral infections. John Wiley and Sons Ltd., West Sussex, England.
11. **Busch, D. H., I. M. Pilip, S. Vijh, and E. G. Pamer.** 1998. Coordinate regulation of complex T cell populations responding to bacterial infection. Immunity **8:**353–362.
12. **Butz, E. A., and M. J. Bevan.** 1998. Differential presentation of the same MHC class I epitopes by fibroblasts and dendritic cells. J. Immunol. **160:** 2139–2144.
13. **Butz, E. A., and M. J. Bevan.** 1998. Massive expansion of antigen-specific CD8+ T cells during an acute virus infection. Immunity **8:**167–175.

14. **Chen, H. D., A. E. Fraire, I. Joris, M. A. Brehm, R. M. Welsh, and L. K. Selin.** 2001. Memory CD8+ T cells in heterologous antiviral immunity and immunopathology in the lung. Nat. Immunol. **2:**1067–1076.
15. **Cho, B. K., C. Wang, S. Sugawa, H. N. Eisen, and J. Chen.** 1999. Functional differences between memory and naive CD8 T cells. Proc. Natl. Acad. Sci. USA **96:**2976–2981.
16. **Crowe, S. R., S. J. Turner, S. C. Miller, A. D. Roberts, R. A. Rappolo, P. C. Doherty, K. H. Ely, and D. L. Woodland.** 2003. Differential antigen presentation regulates the changing patterns of CD8+ T cell immunodominance in primary and secondary influenza virus infections. J. Exp. Med. **198:**399–410.
17. **Davenport, M. P., C. Fazou, A. J. McMichael, and M. F. Callan.** 2002. Clonal selection, clonal senescence, and clonal succession: the evolution of the T cell response to infection with a persistent virus. J. Immunol. **168:**3309–3317.
18. **Erickson, A. L., Y. Kimura, S. Igarashi, J. Eichelberger, M. Houghton, J. Sidney, D. McKinney, A. Sette, A. L. Hughes, and C. M. Walker.** 2001. The outcome of hepatitis C virus infection is predicted by escape mutations in epitopes targeted by cytotoxic T lymphocytes. Immunity **15:**883–895.
19. **Fuller, M. J., A. Khanolkar, A. E. Tebo, and A. J. Zajac.** 2004. Maintenance, loss, and resurgence of T cell responses during acute, protracted, and chronic viral infections. J. Immunol. **172:**4204–4214.
20. **Fuller, M. J., and A. J. Zajac.** 2003. Ablation of CD8 and CD4 T cell responses by high viral loads. J. Immunol. **170:**477–486.
21. **Fuller-Pace, F. V., and P. J. Southern.** 1988. Temporal analysis of transcription and replication during acute infection with lymphocytic choriomeningitis virus. Virology **162:**260–263.
22. **Gallimore, A., T. Dumrese, H. Hengartner, R. M. Zinkernagel, and H. G. Rammensee.** 1998. Protective immunity does not correlate with the hierarchy of virus-specific cytotoxic T cell responses to naturally processed peptides. J. Exp. Med. **187:**1647–1657.
23. **Gallimore, A., H. Hengartner, and R. Zinkernagel.** 1998. Hierarchies of antigen-specific cytotoxic T-cell responses. Immunol. Rev. **164:**29–36.
24. **Goldrath, A. W., and M. J. Bevan.** 1999. Selecting and maintaining a diverse T-cell repertoire. Nature **402:**255–262.
25. **Goulder, P. J., M. A. Altfeld, E. S. Rosenberg, T. Nguyen, Y. Tang, R. L. Eldridge, M. M. Addo, S. He, J. S. Mukherjee, M. N. Phillips, M. Bunce, S. A. Kalams, R. P. Sekaly, B. D. Walker, and C. Brander.** 2001. Substantial differences in specificity of HIV-specific cytotoxic T cells in acute and chronic HIV infection. J. Exp. Med. **193:**181–194.
26. **Grayson, J. M., L. E. Harrington, J. G. Lanier, E. J. Wherry, and R. Ahmed.** 2002. Differential sensitivity of naive and memory CD8+ T cells to apoptosis in vivo. J. Immunol. **169:**3760–3770.
27. **Gruters, R. A., C. A. van Baalen, and A. D. Osterhaus.** 2002. The advantage of early recognition of HIV-infected cells by cytotoxic T-lymphocytes. Vaccine **20:**2011–2015.
28. **Gudmundsdottir, H., A. D. Wells, and L. A. Turka.** 1999. Dynamics and requirements of T cell clonal expansion in vivo at the single-cell level: effector function is linked to proliferative capacity. J. Immunol. **162:**5212–5223.
29. **Hamilton, S. E., B. B. Porter, K. A. Messingham, V. P. Badovinac, and J. T. Harty.** 2004. MHC class Ia-restricted memory T cells inhibit expansion of a nonprotective MHC class Ib (H2-M3)-restricted memory response. Nat. Immunol. **5:**159–168.
30. **Harrington, L. E., M. Galvan, L. G. Baum, J. D. Altman, and R. Ahmed.** 2000. Differentiating between memory and effector CD8 T cells by altered expression of cell surface O-glycans. J. Exp. Med. **191:**1241–1246.
31. **Hislop, A. D., N. E. Annels, N. H. Gudgeon, A. M. Leese, and A. B. Rickinson.** 2002. Epitope-specific evolution of human CD8(+) T cell responses from primary to persistent phases of Epstein-Barr virus infection. J. Exp. Med. **195:**893–905.
32. **Homann, D., L. Teyton, and M. B. Oldstone.** 2001. Differential regulation of antiviral T-cell immunity results in stable CD8+ but declining CD4+ T-cell memory. Nat. Med. **7:**913–919.
33. **Hudrisier, D., M. B. Oldstone, and J. E. Gairin.** 1997. 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. Virology **234:**62–73.
34. **Jiang, J., L. L. Lau, and H. Shen.** 2003. Selective depletion of nonspecific T cells during the early stage of immune responses to infection. J. Immunol. **171:**4352–4358.
35. **Kaech, S. M., and R. Ahmed.** 2001. Memory CD8+ T cell differentiation: initial antigen encounter triggers a developmental program in naive cells. Nat. Immunol. **2:**415–422.
36. **Khanolkar, A., M. J. Fuller, and A. J. Zajac.** 2004. CD4 T cell-dependent CD8 T cell maturation. J. Immunol. **172:**2834–2844.
37. **Klenerman, P., and R. M. Zinkernagel.** 1998. Original antigenic sin impairs cytotoxic T lymphocyte responses to viruses bearing variant epitopes. Nature **394:**482–485.
38. **Klonowski, K. D., K. J. Williams, A. L. Marzo, D. A. Blair, E. G. Lingenheld, and L. Lefrancois.** 2004. Dynamics of blood-borne CD8 memory T cell migration in vivo. Immunity **20:**551–562.
39. **Lalvani, A., R. Brookes, S. Hambleton, W. J. Britton, A. V. Hill, and A. J. McMichael.** 1997. Rapid effector function in CD8+ memory T cells. J. Exp. Med. **186:**859–865.
40. **Lechner, F., D. K. Wong, P. R. Dunbar, R. Chapman, R. T. Chung, P. Dohrenwend, G. Robbins, R. Phillips, P. Klenerman, and B. D. Walker.** 2000. Analysis of successful immune responses in persons infected with hepatitis C virus. J. Exp. Med. **191:**1499–1512.
41. **Lewicki, H., A. Tishon, P. Borrow, C. F. Evans, J. E. Gairin, K. M. Hahn, D. A. Jewell, I. A. Wilson, and M. B. Oldstone.** 1995. CTL escape viral variants. I. Generation and molecular characterization. Virology **210:**29–40.
42. **Liu, F., J. L. Whitton, and M. K. Slifka.** 2004. The rapidity with which virus-specific CD8+ T cells initiate IFN-gamma synthesis increases markedly over the course of infection and correlates with immunodominance. J. Immunol. **173:**456–462.
43. **Moskophidis, D., F. Lechner, H. Pircher, and R. M. Zinkernagel.** 1993. Virus persistence in acutely infected immunocompetent mice by exhaustion of antiviral cytotoxic effector T cells. Nature **362:**758–761.
44. **Murali-Krishna, K., J. D. Altman, M. Suresh, D. J. Sourdive, A. J. Zajac, J. D. Miller, J. Slansky, and R. Ahmed.** 1998. Counting antigen-specific CD8 T cells: a reevaluation of bystander activation during viral infection. Immunity **8:**177–187.
45. **O'Connor, D. H., T. M. Allen, T. U. Vogel, P. Jing, I. P. DeSouza, E. Dodds, E. J. Dunphy, C. Melsaether, B. Mothe, H. Yamamoto, H. Horton, N. Wilson, A. L. Hughes, and D. I. Watkins.** 2002. Acute phase cytotoxic T lymphocyte escape is a hallmark of simian immunodeficiency virus infection. Nat. Med. **8:**493–499.
46. **Pantaleo, G., H. Soudeyns, J. F. Demarest, M. Vaccarezza, C. Graziosi, S. Paolucci, M. Daucher, O. J. Cohen, F. Denis, W. E. Biddison, R. P. Sekaly, and A. S. Fauci.** 1997. Evidence for rapid disappearance of initially expanded HIV-specific CD8+ T cell clones during primary HIV infection. Proc. Natl. Acad. Sci. USA **94:**9848–9853.
47. **Probst, H. C., K. Tschannen, A. Gallimore, M. Martinic, M. Basler, T. Dumrese, E. Jones, and M. F. van den Broek.** 2003. Immunodominance of an antiviral cytotoxic T cell response is shaped by the kinetics of viral protein expression. J. Immunol. **171:**5415–5422.
48. **Pudney, V. A., A. M. Leese, A. B. Rickinson, and A. D. Hislop.** 2005. CD8+ immunodominance among Epstein-Barr virus lytic cycle antigens directly reflects the efficiency of antigen presentation in lytically infected cells. J. Exp. Med. **201:**349–360.
49. **Rodriguez, F., S. Harkins, M. K. Slifka, and J. L. Whitton.** 2002. Immunodominance in virus-induced $CD8^+$ T-cell responses is dramatically modified by DNA immunization and is regulated by gamma interferon. J. Virol. **76:**4251–4259.
50. **Seder, R. A., and R. Ahmed.** 2003. Similarities and differences in CD4+ and CD8+ effector and memory T cell generation. Nat. Immunol. **4:**835–842.
51. **Slifka, M. K., F. Rodriguez, and J. L. Whitton.** 1999. Rapid on/off cycling of cytokine production by virus-specific CD8+ T cells. Nature **401:**76–79.
52. **Steven, N. M., A. M. Leese, N. E. Annels, S. P. Lee, and A. B. Rickinson.** 1996. Epitope focusing in the primary cytotoxic T cell response to Epstein-Barr virus and its relationship to T cell memory. J. Exp. Med. **184:**1801–1813.
53. **Stittelaar, K. J., R. A. Gruters, M. Schutten, C. A. van Baalen, G. van Amerongen, M. Cranage, P. Liljestrom, G. Sutter, and A. D. Osterhaus.** 2002. Comparison of the efficacy of early versus late viral proteins in vaccination against SIV. Vaccine **20:**2921–2927.
54. **Tripp, R. A., S. Hou, and P. C. Doherty.** 1995. Temporal loss of the activated L-selectin-low phenotype for virus-specific CD8+ memory T cells. J. Immunol. **154:**5870–5875.
55. **Turner, S. J., G. Diaz, R. Cross, and P. C. Doherty.** 2003. Analysis of clonotype distribution and persistence for an influenza virus-specific CD8+ T cell response. Immunity **18:**549–559.
56. **van Baalen, C. A., C. Guillon, M. van Baalen, E. J. Verschuren, P. H. Boers, A. D. Osterhaus, and R. A. Gruters.** 2002. Impact of antigen expression kinetics on the effectiveness of HIV-specific cytotoxic T lymphocytes. Eur. J. Immunol. **32:**2644–2652.
57. **van Baalen, C. A., O. Pontesilli, R. C. Huisman, A. M. Geretti, M. R. Klein, F. de Wolf, F. Miedema, R. A. Gruters, and A. D. Osterhaus.** 1997. Human immunodeficiency virus type 1 Rev- and Tat-specific cytotoxic T lymphocyte frequencies inversely correlate with rapid progression to AIDS. J. Gen. Virol. **78:**1913–1918.
58. **van der Most, R. G., K. Murali-Krishna, J. G. Lanier, E. J. Wherry, M. T. Puglielli, J. N. Blattman, A. Sette, and R. Ahmed.** 2003. Changing immunodominance patterns in antiviral CD8 T-cell responses after loss of epitope presentation or chronic antigenic stimulation. Virology **315:**93–102.
59. **van der Most, R. G., K. Murali-Krishna, J. L. Whitton, C. Oseroff, J. Alexander, S. Southwood, J. Sidney, R. W. Chesnut, A. Sette, and R. Ahmed.** 1998. Identification of Db- and Kb-restricted subdominant cytotoxic T-cell responses in lymphocytic choriomeningitis virus-infected mice. Virology **240:**158–167.
60. **van Stipdonk, M. J., E. E. Lemmens, and S. P. Schoenberger.** 2001. Naive CTLs require a single brief period of antigenic stimulation for clonal expansion and differentiation. Nat. Immunol. **2:**423–429.
61. **Veiga-Fernandes, H., U. Walter, C. Bourgeois, A. McLean, and B. Rocha.**

2000. Response of naive and memory CD8+ T cells to antigen stimulation in vivo. Nat. Immunol. **1:**47–53.
62. **Welsh, R. M.** 2000. Lymphocytic choriomeningitis virus as a model for the study of cellular immunology, p. 289–312. *In* M. W. Cunningham and R. S. Fujinami (ed.), Effects of microbes on the immune system. Lippincott Williams and Wilkins, Philadelphia, Pa.
63. **Welsh, R. M., L. K. Selin, and E. Szomolanyi-Tsuda.** 2004. Immunological memory to viral infections. Annu. Rev. Immunol. **22:**711–743.
64. **Wherry, E. J., and R. Ahmed.** 2004. Memory CD8 T-cell differentiation during viral infection. J. Virol. **78:**5535–5545.
65. **Wherry, E. J., J. N. Blattman, K. Murali-Krishna, R. van der Most, and R. Ahmed.** 2003. Viral persistence alters CD8 T-cell immunodominance and tissue distribution and results in distinct stages of functional impairment. J. Virol. **77:**4911–4927.
66. **Whitmire, J. K., J. T. Tan, and J. L. Whitton.** 2005. Interferon-gamma acts directly on CD8+ T cells to increase their abundance during virus infection. J. Exp. Med. **201:**1053–1059.
67. **Yewdell, J. W., and J. R. Bennink.** 1999. Immunodominance in major histocompatibility complex class I-restricted T lymphocyte responses. Annu. Rev. Immunol. **17:**51–88.