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Kapil Bahl,* Sung-Kwon Kim, Claúdia Calcagno, Dario Gherzi, Roberto Puzone, Franco Celada, Liisa K. Selin,* and Raymond M. Welsh†*†

Profound lymphopenia has been observed during many acute viral infections, and our laboratory has previously documented a type I IFN-dependent loss of CD8 T cells immediately preceding the development of the antiviral T cell response. Most memory (CD44high) and some naive (CD44low) CD8 T cells are susceptible to IFN-induced attrition, and we show in this study that the IFN-induced attrition of CD8+CD44high T cells is associated with elevated activation of caspase-3 and caspase-8. We questioned whether TCR engagement by Ag would render CD8 T cells resistant to attrition. We tested whether a high concentration of Ag (GP33 peptide) would protect lymphocytic choriomeningitis (LCMV)-specific naive CD8 T cells (TCR transgenic P14 cells specific for the GP33 epitope of LCMV) and memory CD8 T cells (GP33-specific LCMV-immune cells) from deletion. Both naive P14 and memory GP33-specific donor CD8 T cells decreased substantially 16 h after inoculation with the Toll receptor agonist and IFN inducer, poly(I:C), regardless of whether a high concentration of GP33 peptide was administered to host mice beforehand. Moreover, donor naive P14 and LCMV-specific memory cells were depleted from day 2 LCMV-infected hosts by 16 h posttransfer. These results indicate that Ag engagement does not protect CD8 T cells from the IFN-induced T cell attrition associated with viral infections. In addition, computer models indicated that early depletion of memory T cells may allow for the generation for a more diverse T cell response to infection by reducing the immunodomination caused by cross-reactive T cells. *The Journal of Immunology, 2006, 176: 4284–4295.

The early stages of infections by many human viruses, including influenza, measles, West Nile, Ebola, Lassa fever, lymphocytic choriomeningitis (LCMV), and SARS coronavirus viruses, are characterized by a severe lymphopenia (1–4). Similar lymphopenias are seen in viral infections of domestic animals (5–8) and in laboratory models of infection. The dynamics of this lymphopenia have been established in mice infected with LCMV, where acute infection leads to a reduction in lymphocyte numbers 2–4 days postinfection, preceding the expansion of LCMV-specific T and B cells and the enlargement of lymphoid organs (2). There is a substantial loss in many types of leukocytes during this early lymphopenia, but bona fide Ag-specific memory and “memory phenotype” (CD44high) CD8 T cells are among the most susceptible. This loss in CD8 T cells occurs throughout the body, including both lymphoid and nonlymphoid tissues, and cannot be accounted for simply by lymphocyte migration. The loss of memory CD8 T cells is, at least in part, due to apoptosis, as they are positive for the early apoptotic marker annexin V and for TUNEL (1, 9, 10).

Although the underlying mechanisms leading to the attrition of memory CD8 T cells are still under investigation, kinetic analyses revealed that type I IFN induction immediately preceded this early attrition (1). Experiments using the potent type I IFN inducer, poly(I:C), caused a similar reduction in total lymphocytes and memory CD8 T cells compared with an LCMV infection, though with more rapid kinetics, in parallel with the IFN response. Type I IFN-deficient mice were resistant to the early attrition, with little reduction in total lymphocytes or memory phenotype CD8 T cells after either LCMV infection or poly(I:C) treatment (1). This loss in T cells can be induced by inoculation with rIFN-α (1) and blocked by Ab to type I IFN (11). Recent studies have also linked the early type I IFN response to impairment in dendritic cell differentiation and function early during the LCMV infection, but it is not known whether these phenomena are related (12).

Many of the viruses associated with profound lymphopenia can also induce remarkably strong immune responses, provided that innate defenses keep the infection from overwhelming the host. This finding, plus the fact that partial lymphopenia induced by sublethal irradiation or immunosuppressive drug treatment can enhance immune responses to tumor (13) and viral (14) Ags, has led us to speculate that the virus-induced lymphopenia might facilitate the mounting of a strong antiviral T cell response (1, 2). Consistent with this hypothesis is the fact that older mice develop less initial lymphopenia and mount a weaker CD8 T cell response to LCMV than do younger mice (15, 16).

Studies have shown that engagement of naive T cells with their ligand, under conditions of appropriate costimulation, causes the up-regulation of the antiapoptosis protein Bcl-xL, thereby giving...
cells a survival advantage (17). One might, therefore, predict that Ag-specific T cells engaging their cognate ligand would preferentially resist the IFN-induced apoptosis. It was recently reported that depletion of both naïve and memory CD8 T cells during the early stages of the immune response to infection is selective (9). Most T cells, regardless of specificity, were induced to express early activation markers upon infection, but nonspecific T cells were depleted, whereas those T cells specific for the pathogen expressed late activation markers and expanded in number (9). However, it is unclear whether there was a transient apoptotic loss before the rapid Ag-specific proliferation.

We report the unexpected finding that Ag engagement of naïve or memory T cells does not prevent this early apoptosis, and by using computer models, we propose how this susceptibility to apoptosis may allow for a more diverse T cell response to infection. Furthermore, we show that the IFN-induced attrition of CD8+ CD44high T cells is associated with a caspase-dependent pathway, involving key initiator (caspase-8) and effector (caspase-3) caspases.

Materials and Methods

Virus stocks and inoculation

LCMV Armstrong strain and its highly disseminating variant, clone 13, are amphisense RNA viruses in the Old World arenavirus family and were propagated in baby hamster kidney BHK21 cells (18, 19). Pichinde virus strain AN3739, a New World arenavirus only distantly related to LCMV, was propagated in BHK21 cells (19). LCMV and Pichinde virus were titrated in MA104 cells and expressed late activation markers and expanded in number (9). However, it is unclear whether there was a transient apoptotic loss before the rapid Ag-specific proliferation.

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Results

Characterization of apoptotic properties of CD8 T cells after poly(I:C) inoculation

It has been previously demonstrated that CD8 T cells undergo apoptosis and decline in number in response to IFN-αβ. This apoptotic cell loss, however, is more pronounced in the memory and “memory phenotype” CD8 T cell population (CD44<sup>high</sup>) than in the naive CD8 T cell population (CD44<sup>low</sup>) (1). A greater than 75% reduction in the number of CD8<sup>+</sup>CD44<sup>high</sup> T cells in the spleen occurred 24 h following poly(I:C) treatment, whereas a smaller (25%), though significant, reduction in CD8<sup>+</sup>CD44<sup>low</sup> T cell number was also observed at this time point following poly(I:C) treatment (Fig. 1A). Although both populations underwent a decline in cell number 12 h following poly(I:C) treatment, the CD8<sup>+</sup>CD44<sup>high</sup> T cell population exhibited a greater increase in annexin V reactivity relative to the CD8<sup>+</sup>CD44<sup>low</sup> T cell population (Fig. 1B), suggesting that the cell loss exhibited by both populations following poly(I:C) treatment might be regulated by different mechanisms downstream of IFN signaling. IFN-αβ receptor-deficient mice exhibited a slight decline in CD8<sup>+</sup>CD44<sup>high</sup> T cell number 24 h following poly(I:C) treatment, but this reduction was not statistically significant (Fig. 1A). No significant decrease in the number of CD8<sup>+</sup>CD44<sup>low</sup> T cells was observed in IFN-αβ receptor-deficient mice 24 h following poly(I:C) treatment (Fig. 1A). Neither IFN-αβ receptor-deficient population (CD8<sup>+</sup>CD44<sup>high</sup> or CD8<sup>+</sup>CD44<sup>low</sup>) had an increase in annexin V reactivity 12 h following poly(I:C) treatment, further supporting the involvement of IFN-αβ in the apoptotic loss of CD8 T cells, particularly in the CD8<sup>+</sup>CD44<sup>high</sup> (Fig. 1B).

Although type I IFN is involved, the downstream molecular mechanisms leading to the apoptosis of CD8<sup>+</sup>CD44<sup>high</sup> T cells have not yet been determined. Previous studies have shown that the IFN-α-induced apoptosis of some malignant cell lines occurred by a caspase-dependent mechanism (33). Caspases are a family of intracellular cysteine proteases that play a central role in the initiation and execution of apoptosis. Caspase-8 is a major initiator caspase, which is activated upon death receptor signaling and can initiate the activation of the caspase cascade via both the death receptor and mitochondrial death pathways (34, 35). Caspase-3 is a downstream effector caspase that serves as the point of convergence of both the death receptor and mitochondrial death pathways. Therefore, we chose to compare the level of caspase-3 and caspase-8 activation at 0, 12, and 24 h post-poly(I:C) inoculation between CD8<sup>+</sup>CD44<sup>low</sup> and CD8<sup>+</sup>CD44<sup>high</sup> T cells from both wild-type (129) and IFN-R KO mice. The level of total caspase activity was determined via a pan-caspase substrate. Overall, caspase activation (caspase-3, caspase-8, and pan-caspase) was higher in CD8<sup>+</sup>CD44<sup>high</sup> T cells than CD8<sup>+</sup>CD44<sup>low</sup> T cells in both 129 and IFN-R KO mice before poly(I:C) treatment (Fig. 1C). There was, however, a slight increase in the caspase activation of 129 CD8<sup>+</sup>CD44<sup>high</sup> T cells relative to that of IFN-R KO CD8<sup>+</sup>CD44<sup>high</sup> T cells at 12 h post-poly(I:C), although this increase was not statistically significant. There was a statistically significant increase in caspase-8 activation of 129 CD8<sup>+</sup>CD44<sup>high</sup> T cells relative to that of IFN-R KO CD8<sup>+</sup>CD44<sup>high</sup> T cells (p < 0.05) 24 h following poly(I:C) treatment, and this correlated with an increase in annexin V reactivity (Fig. 1B). Total caspase and caspase-3 activation of 129 CD8<sup>+</sup>CD44<sup>high</sup> T cells relative to that of IFN-R KO CD8<sup>+</sup>CD44<sup>high</sup> T cells were also higher, although not statistically significant (p < 0.06). There was no significant increase in the caspase activation of either wild-type or IFN-R KO CD8<sup>+</sup>CD44<sup>low</sup> T cells upon poly(I:C) treatment. Thus, the increase in overall caspase activity (pan-caspase), along with caspase-3 and caspase-8 activation, correlated with apoptotic loss of wild-type CD8<sup>+</sup>CD44<sup>high</sup> T cells.

GP33–45 peptide does not protect naive Ag-specific CD8 T cells from depletion induced by poly(I:C)

A previous study has reported that adoptively transferred TCR Tg P14 T cells, specific for the GP33 epitope of LCMV, were protected from depletion when measured 3 days after LCMV infection, whereas OT-1 Tg T cells, specific for OVA, were significantly depleted, suggesting that TCR engagement by Ag may protect Ag-specific cell depletion (9). However, it was unclear whether this reflected a resistance to early depletion or a compensatory proliferation after the depletion event had occurred. We, therefore, designed a system to examine the early apoptosis by administering Ag before the induction of cytokine-induced lymphopenia. Therefore, to determine whether Ag engagement protects Ag-specific T cells from depletion, P14 splenocytes (Ly5.2<sup>+</sup>) and OT-1 splenocytes (Thy1.1<sup>+</sup>) were labeled with CFSE and adoptively transferred into naive B6 recipient mice (Ly5.1<sup>+</sup>). Ly5.1<sup>+</sup> recipient mice were then inoculated with GP33–45 peptide (i.v.) or PBS (i.v.), 1 day after transfer. Immunization with GP33–45 peptide alone has been shown to elicit CTL responses in vivo (25), making it an appropriate Ag for use in our model system. This concentration of peptide and inoculation procedure for P14 cells has been optimized by others in our department, and has been shown to result in the proliferation of virtually all (>95%) detectable CFSE-labeled P14 cells by 3 days postpeptide inoculation (36). In our model, >70% of the P14 Tg cells had up-regulated CD69 expression by 21 h postpeptide treatment (Fig. 2A) and no division had yet occurred, as indicated by the lack of CFSE dilution (Fig. 2B). These results indicate that there is sufficient GP33 peptide available to engage the receptors on the P14 T cells. Moreover, this dose of peptide does not result in the proliferation of P14 donor cells between the time of initial peptide inoculation and splenocyte harvest (21 h). Both P14 and OT-1 CD8 donor cells had low levels of CD44 expression (<15%). Five hours after the GP33–45 peptide was administered, recipient mice were inoculated i.p. with either poly(I:C) or PBS. Splenocytes were harvested 16 h after poly(I:C) inoculation. Previous experiments using BrdU to determine cell division showed no increase in labeling with BrdU by CD8<sup>+</sup> T cells 24 h following poly(I:C) treatment, suggesting that there was no cell division immediately following IFN-αβ treatment (1). Therefore, this system allows us to study whether TCR engagement by Ag (GP33 peptide) may protect Ag-specific cells (P14 cells) from depletion without the potential for compensatory proliferation to occur.

Donor splenocytes (combined P14 and OT-1 cells) represented 0.5% of the untreated host splenocyte population. In the experiment shown, OT-1 cells comprised 60%, whereas P14 cells comprised 35% of the donor population, as distinguished by Thy1.2 and Vα2 staining (Fig. 2B). The percentage of donor splenocytes decreased by half (to 0.24%) after poly(I:C) treatment, but the ratio of the Tg T cells in the donor population changed only slightly (OT-1 to P14 ratio was 54 to 41%, respectively), suggesting that both Tg T cell populations were equally susceptible to depletion. Inoculation with GP33–45 peptide alone had little effect on the percentage of donor splenocytes (0.52%) or on the ratio of OT-1 to P14 donor cells (57 to 38%) in the donor population. After poly(I:C) inoculation of GP33–45 peptide-treated host mice, there was a substantial loss in the donor splenocyte population (0.35%), which was not as great as without peptide (0.24%). Again, the ratio of the two Tg populations changed slightly (OT-1 to P14 ratio was 61 to 31%, respectively). There was, however, a statistically significant reduction in the percentage of P14 cells with GP33–45...
FIGURE 1. The IFN-induced apoptosis of memory-phenotype CD8 T cells (CD44\textsuperscript{high}) is associated with caspase activation. Wild-type (129) or IFN-R KO mice were inoculated with poly(l:C) (i.p.). Splenocytes were harvested 0, 12, and 24 h postinoculation. A. Absolute numbers of CD8\textsuperscript{+}CD44\textsuperscript{high} and CD8\textsuperscript{+}CD44\textsuperscript{low} splenocytes present 0, 12, and 24 h post-poly(I:C) treatment. Results were calculated based on percentages obtained via flow cytometry. Representative data with \( n = 3 \) mice per group. \( * \), \( p < 0.05 \) of wild-type at 24 h post-poly(I:C) treatment compared with 0 h control. B. Percentages of annexin V\textsuperscript{+}CD8\textsuperscript{+}CD44\textsuperscript{high} and CD8\textsuperscript{+}CD44\textsuperscript{high} wild-type (129) and IFN-R KO post-poly(I:C) treatment. Gates were set upon early apoptotic (7-AAD\textsuperscript{-}) CD8\textsuperscript{+} cells to exclude dead/necrotic cells. C. Mean fluorescent intensity (MFI) of caspase activity (caspase-3, caspase-8, and pan-caspase) for both wild-type (129) and IFN-R KO CD8\textsuperscript{+}CD44\textsuperscript{high} and CD8\textsuperscript{+}CD44\textsuperscript{low} T cells 0, 12, and 24 h post-poly(I:C) treatment. \( * \), \( p < 0.05 \) of wild-type at 24 h post-poly(I:C) treatment compared with 0 h control. Representative data (B) are shown with \( n = 3 \) mice per group (A). The data are representative of two independent experiments.
peptide followed by poly(I:C) inoculation when compared with poly(I:C) alone, as indicated by the relative decrease in the percentage of P14 cells (31 vs 41%, respectively; \( p = 0.01 \)) (Fig. 2B). Moreover, despite the GP33–45 peptide-induced increased activation status (mean fluorescent intensity of CD69 expression) of P14 cells relative to OT-1 T cells, this status did not correlate with an increase rate of survival after poly(I:C) treatment (19,349 vs 9676, respectively) (Fig. 2A).

Fig. 2B shows a representative experiment showing changes in percentage of donor T cell populations within a host’s splenocyte population, but this does not take into consideration the global effects of lymphopenia on total (host and donor) cell populations. Fig. 2C depicts the average splenocyte numbers of several mice per group after poly(I:C) treatment. P14 donors were reduced by \( \sim 50\% \), whereas OT-1 cells were reduced by \( \sim 66\% \) upon poly(I:C) inoculation. The greater decrease in the number of OT-1 cells relative to the number of P14 cells was unexpected and may be due to different susceptibilities of the Tg cells to IFN-induced depletion. When GP33–45 peptide was administered before poly(I:C) inoculation, P14 cells were reduced by \( \sim 66\% \), whereas OT-1 cells were still reduced by \( \sim 60\% \). There was a difference between the number of P14 cells remaining after GP33–45 peptide and poly(I:C) treatment vs poly(I:C) treatment, although not quite significant (\( p = 0.06 \)) (Fig. 2C). Combined, these results suggest that GP33–45 peptide may slightly enhance depletion rather than protect the P14 cells from depletion. Overall, these findings indicate that GP33–45 peptide was unable to prevent the depletion of P14 cells during poly(I:C)-induced lymphopenia.
Ag-specific naive CD8 T cells are not protected from depletion during the early phase of an acute viral infection

We next examined whether naive Ag-specific CD8 T cells are protected from depletion during the early stages of an acute viral infection. To increase the probability of Ag engagement of the T cells, we used the highly disseminating variant of LCMV, clone 13, which differs from the Armstrong strain by two amino acids, but encodes similar T cell epitopes (37). We chose day 2 post-infection because both IFN levels and viral load are high at this time point (38-40), and a decline of CD8 T cell numbers had been detected as early as 2 days post-LCMV infection (41). CFSE-labeled P14 cells (Ly5.2) were transferred (i.v.) into either a day 2 clone 13-infected mouse or a naive C57BL/6 mouse (Ly5.1). Splenocytes were harvested 16 h later. There was a 10-fold loss in the percentage of donor P14 cells when transferred into a day 2 clone 13-infected mouse upon harvest (Fig. 3A). This loss of donor P14 cells was not specific to the spleen, as the loss also occurred in the lymph nodes (Fig. 3A), peripheral blood, bone marrow, peritoneal cavity, and lungs (data not shown), ruling out trafficking to other compartments.

Decreases in the number of P14 donor splenocytes transferred into a day 2 clone 13-infected mouse were consistent with the decrease in the frequency of total P14 donor cells. Transfer into a day 2 clone 13-infected mouse resulted in a >90% reduction in the number of P14 donor cells, relative to transfer into a naive recipient (Fig. 3B). Host CD8 T cells (Ly5.1) underwent a 70% reduction 2 days post-clone 13 infection, relative to a naive Ly5.1 mouse (Fig. 3B). These results suggest that the high viral Ag load does not prevent the depletion of Ag-specific naive P14 cells during the early lymphopenic phase of an acute LCMV infection.

GP33–45 peptide does not protect Ag-specific memory CD8 T cells from depletion by poly(I:C) treatment

Although both naive and memory CD8 T cells are susceptible to the early apoptosis phenomenon, bona fide Ag-specific memory and “memory phenotype” CD8\(^+\)CD44\(^{high}\) T cells are far more susceptible (Fig. 4A) (1). Because memory cells react with ligand in qualitatively and quantitatively different ways than naive cells (42–45), we also questioned whether Ag engagement would protect them from the early apoptosis. We examined whether treatment with GP33–45 peptide selectively protected GP33-specific, but not other LCMV-specific memory cells, from poly(I:C)-induced depletion. CFSE-labeled LCMV-immune splenocytes (Ly5.2) were adoptively transferred into naive B6 mice (Ly5.1). Ly5.1 recipient mice were then inoculated with GP33–45 peptide (i.v.) or PBS (i.v.) 1 day after transfer. After 5 h, recipient mice were inoculated with poly(I:C) (i.p.). Splenocytes were harvested 16 h post-poly(I:C) inoculation. As expected, both naive recipient (Ly5.1) and LCMV-immune donor (Ly5.2) CD8 T cell populations underwent attrition upon poly(I:C) inoculation of otherwise untreated mice (92 and 85% reduction, respectively), with a greater decrease in the CD44\(^{high}\) relative to the CD44\(^{low}\) population (Fig. 4A). GP33–45 peptide inoculation resulted in a slight reduction in the frequency of LCMV-immune donor (Ly5.2)

**FIGURE 3.** High viral load does not protect Ag-specific naive CD8 T cells from depletion during the early phase of an acute viral infection. A total of 3–4 × 10\(^6\) CFSE-labeled P14 Tg T cells (Ly5.2) were transferred into either a LCMV clone 13-infected (day 2) mice or a naive C57BL/6 mouse (Ly5.1). Splenocytes were harvested 16 h post-transfer. A. The percentage of P14 donors (Ly5.2) and recipient CD8 T cells (Ly5.1) present in both the pooled lymph nodes (axillary, brachial, cervical, and inguinal lymph nodes) and spleen populations is shown. B. Absolute numbers of P14 donors splenocytes present 16 h post-transfer into either LCMV clone 13-infected (day 2) mice or naive C57BL/6 mice (Ly5.1). Numbers were calculated based on percentages obtained from the flow cytometry plots in A. Representative experiment of \(n = 3\) mice per group. The data are representative of two independent experiments.
CD8^+^CD44^{high} T cells relative to no treatment and did not protect against poly(I:C)-induced attrition. GP33–45 peptide treatment followed by poly(I:C) inoculation resulted in a similar degree of attrition in both recipient CD44^{high} (Ly5.1) and LCMV-immune donor (Ly5.2) CD8 T cells compared with poly(I:C) treatment only (Fig. 4).

Decreases in GP33-specific CD8 T cell numbers upon peptide and/or poly(I:C) treatment were consistent with the decreases in the frequencies of total LCMV-specific immune donor cells (Fig. 4B). Poly(I:C) treatment resulted in a 67% reduction in the number of GP33-specific CD8 memory T cells and a 51% reduction in the number of total memory CD8 T cells, as determined by anti-CD3 stimulation. GP33–45 peptide treatment resulted in almost a 44% decrease in the number of GP33-specific CD8 memory T cells relative to untreated controls and only a marginal 13% decrease in the total number of memory CD8 T cells. GP33–45 peptide treatment followed by poly(I:C) inoculation resulted in an 80% decrease in the number of GP33-specific CD8 memory T cells, relative to untreated controls. The total number of bona fide memory CD8 T cells underwent a similar degree of attrition regardless of whether or not the GP33–45 peptide was administered before poly(I:C) treatment (Fig. 4B). Overall, these findings indicate that GP33–45 peptide was unable to prevent the depletion of GP33-specific CD8 memory T cells during poly(I:C)-induced lymphopenia and may enhance, rather than protect against depletion (Fig. 4).

Ag-specific memory CD8 T cells are not protected from depletion during the early phase of an acute viral infection

To test whether LCMV-specific memory CD8 T cells were protected from depletion during the attrition phase of the immune response to a viral infection, CFSE-labeled LCMV-immune cells (Ly5.2) were transferred (i.v.) into a day 2 clone 13-infected host, day 2 Armstrong-infected host, or a naive host (Ly5.1). As a non-specific control, Pichinde virus-specific memory CD8 T cells were also transferred under the same conditions. Splenocytes were harvested 16 h later.

Both LCMV- and Pichinde virus-immune donor CD8 T cells underwent almost a 50% reduction in both LCMV clone 13- and
LCMV Armstrong-infected mice (Fig. 5A). There was almost a complete loss of LCMV-specific (NP396, GP33, and GP276) and Pichinde virus-specific (NP38) CD8 memory T cells after they were transferred into either an Armstrong- or clone 13-infected mouse (2 days postinfection) (Fig. 5C). The loss of donor memory cells was not due to trafficking out of the spleen, as other compartments experienced a similar donor cell loss, including the lymph nodes (Fig. 5B), peripheral blood (0.1 to <0.01%), and lungs (0.1–0.04%) 16 h posttransfer clone 13-infected mice. These results suggest that the high viral Ag load does not prevent the depletion of LCMV-specific memory CD8 T cells during the early lymphopenic phase of an acute LCMV infection.

**FIGURE 5.** High viral load does not protect Ag-specific memory CD8 T cells from depletion during the early phase of an acute viral infection. A total of $4 \times 10^7$ CFSE-labeled LCMV-immune cells or Pichinde virus (PV)-immune cells (Ly5.2) were transferred into a LCMV Armstrong-infected host (day 2), a LCMV clone 13-infected host (day 2), and a naive host (Ly5.1). Splenocytes were harvested 16 h posttransfer. Donor CD8 T cells were visualized via an IFN-γ assay. A, Percentages of both LCMV and Pichinde virus donor (Ly5.2) CD8 T cells as well as recipient (Ly5.1) CD8 T cells present posttreatment. B, Percentages of LCMV donor (Ly5.2) CD8 T cells as well as recipient (Ly5.1) CD8 T cells present in the inguinal lymph nodes (LN) 16 h posttransfer. C, Absolute numbers of donor LCMV-specific and Pichinde virus-specific memory CD8 T cells present posttreatment. Results were calculated based on percentages obtained from the flow cytometry plots in A. Representative data are shown with $n = 3$ mice/group. The data are representative of two independent experiments.
Computer modeling indicates that early apoptosis of memory cells allows for more diversity in newly arising T cell responses

Computer simulations were used to determine the impact of virus-induced lymphopenia on the T cell response to a virus infection in virtual mice that had a partially cross-reactive pool of memory T cells. The simulations first showed that, in the absence of a memory cell lymphopenia, cross-reactive T cells would dominate a new T cell response and inhibit the emergence of a complex naive T cell response specific to a new pathogen. We then tested the hypothesis that early lymphopenia of memory T cells would facilitate the development of a more diverse naive T cell response.

The simulation tests that, after a successful interaction with an APC, the T cell is protected from IFN type I-mediated death for a designated number of time steps, decided at the beginning of the simulation. Thus, during the heterologous virus infection, only the cross-reactive memory cell clones will be protected from apoptosis, whereas the non-cross-reactive ones will die. This result is contrasted with the model by which even the cross-reactive clones undergo apoptosis.

A virtual mouse was challenged with 70 particles of virus, which was cleared before time step 500, thanks to an effective immune response. The CD8 memory T cells were transferred into different virtual recipients, and a second challenge with 240 particles of a partially cross-reactive virus was administered. The larger amount of challenge virus was necessary to elicit a valid response because of the cross-reactive CD8 T cell pool. The two simulations modeled were 1) specific protection from active attrition conferred to cross-reactive clones (Fig. 6A) and 2) absence of any kind of protection from attrition (Fig. 6B). In this model a low affinity cross-reactive clone is depicted in red. The y-axis shows its frequency before infection. In the absence of attrition, this low-affinity clone can dominate the immune response against high- and medium-affinity clones (green and blue) originating at low frequency from a naive memory pool (Fig. 6). Fig. 6B shows that, if this low-affinity cross-reactive clone undergoes apoptosis, an immunodominant response can be generated by higher-affinity clones (i.e., green) from the naive repertoire.

**Discussion**

A profound lymphopenia occurs before the induction of the T cell response in many viral infections (1–4). This report re-addresses the concept of CD8 T cell attrition under conditions of viral infection or TLR agonist (poly(I:C)) exposure (1, 9) and newly documents a T cell loss and IFN-dependent apoptosis associated with elevated levels of caspase-8, and confirms a previous finding of elevated levels of caspase-3 (11). The most dramatic levels of T cell attrition, apoptosis, and caspase activation were in memory phenotype (CD44high) CD8 T cells, though naive phenotype CD8 T cells were also affected (Fig. 1). Although the peak level of CD8+CD44high T cell loss and caspase activation occurs 24 h following poly(I:C) treatment, the peak level of apoptosis, as indicated by increased annexin V reactivity, occurs 12 h following poly(I:C) treatment. Therefore, the cells remaining at 24 h following poly(I:C) treatment represent the CD8+CD44high T cells that...
have survived the early apoptotic phase because this is the time point at which cell loss peaks and annexin V reactivity is low. The CD8\(^+\) CD44\(^{high}\) T cells that survive this early apoptosis will begin to proliferate in 24 h (48 h following poly(I:C) treatment), and because caspase-8 activation is also associated with proliferation (46–49), it is not surprising that the level of caspase-8 activation continues to increase at 24 h following poly(I:C) treatment. Very little T cell attrition, lymphopenia, or elevated caspase activation occurred in mice lacking type I IFNRs (Fig. 1). A recent report in a similar system showed that CD8\(^+\) T cell attrition could be inhibited by treating mice with Ab to type I IFN, confirming its role in this process (11).

We show that Ag engagement by Ag-specific naive (P14) or memory (LCMV-immune) CD8\(^+\) T cells with either peptide or viral Ag does not protect these cells from depletion resulting from poly(I:C) inoculation or viral infection (LCMV). This result is in contrast to an earlier report that concluded that depletion was selective for nonspecific CD8\(^+\) T cells and that Ag-specific CD8\(^+\) T cells resisted deletion and instead underwent extensive proliferation (9). We suggest that this report may have missed an early depletion preceding the proliferation phase. Our study also seems at odds with the concept that Ag engagement can up-regulate anode depletion preceding the proliferation phase. Our study also seems contrast to an earlier report that concluded that depletion was selective for nonspecific CD8\(^+\) T cells and that Ag-specific CD8\(^+\) T cells resisted depletion and instead underwent extensive proliferation (9). We suggest that this report may have missed an early depletion preceding the proliferation phase. Our study also seems at odds with the concept that Ag engagement can up-regulate antiapoptotic proteins such as Bcl-x\(_L\) and protect cells from apoptosis (50). Our results indicate that protection from apoptosis does not occur at these very early stages of the IFN response. Poly(I:C) treatment up-regulated CD69 on both donor naive P14 and OT-1 Tg T cell populations (Fig. 2A), consistent with the ability of type I IFN to nonspecifically up-regulate CD69 on naive T cells (51, 52). In contrast, GP33–45 peptide up-regulated CD69 only on the GP33-specific P-14 T cells, but not on the OVA-specific OT-1 cells (Fig. 2A). In addition, GP33–45 peptide inoculation down-regulated CD62L expression on P14 cells, but not on OT-1 cells (data not shown). These experiments show the nonspecific effects of the Toll agonist poly(I:C) and the very specific effects of peptide on the Tg T cell populations. This peptide-dependent engagement of the T cells did not, however, selectively protect them from the apoptosis and attrition mediated by poly(I:C) (Fig. 2). Therefore, the increased activation status of naive Ag-specific cells via peptide inoculation did not confer resistance to IFN-induced depletion.

Memory CD8\(^+\) T cells are more susceptible than naive CD8\(^+\) T cells to IFN-induced depletion (1) (Fig. 4A). Because the experiments with Tg P14 T cells used immunologically naive T cells, we also investigated whether the GP33–45 peptide would protect GP33-specific memory CD8\(^+\) T cells from poly(I:C)-induced depletion. However, under conditions of peptide treatment the decrease in GP33-specific memory CD8\(^+\) T cells was similar to that of the decrease in total memory-phenotype CD8\(^+\) T cells and T cells specific to other LCMV epitopes tested (Fig. 4B and data not shown). Treatment with GP33–45 peptide alone resulted in a slight loss of GP33-specific memory CD8\(^+\) T cells, suggesting that memory CD8\(^+\) T cells may be more susceptible to activation-induced cell death than naive CD8\(^+\) T cells (Fig. 4B).

Similar results showing a failure of Ag to protect T cells from deletion were found in virus-infected mice. When naive Tg P14 cells or LCMV-specific memory CD8\(^+\) T cells were transferred into day 2 LCMV clone 13-infected mice, where substantial levels of Ag should be present, the donor P14 cells were significantly depleted (>90%) 16 h posttransfer (Fig. 3). Serum from day 2 LCMV clone 13-infected mice and poly(I:C)-treated mice, 16 h postinfection, had comparable IFN endpoint dilution titers of 1/6400 in a standard type I IFN bioassay using microtiter plates of L-929 cells challenged with vesicular stomatitis virus. LCMV-specific memory CD8\(^+\) T cells also underwent a significant (~50%) depletion when transferred into a day 2 post-clone 13-infected mouse, a result comparable to the rate of depletion when Pichinde virus-specific CD8\(^+\) T cells were transferred into a day 2 clone 13-infected mouse (60% reduction) (Fig. 5A). This decrease occurred in T cells specific to all epitopes tested (NP396, GP33, GP276, and NP38) (Fig. 5C). Thus, Ag load has no protective effect on this early T cell attrition and, if anything, may enhance the attrition. The loss of memory CD8\(^+\) T cells could not be attributed to trafficking into other organs, such as the peripheral blood, bone marrow, peritoneal cavity, lungs, or lymph nodes (Fig. 5B and data not shown). Collectively, these results show that Ag-specific naive and memory CD8\(^+\) T cells are not protected from depletion during the early stages of the immune response to a viral infection.

CD8\(^+\) T cell memory to virus is stable in the absence of infection, but apoptotic events that occur during the early immune response to infection can leave the host with a reduced population of memory CD8\(^+\) T cells to previously encountered viruses (53–55). Although this early depletion may appear to be detrimental to the host, studies in which a lymphopenic state was induced via cytotoxic drugs or irradiation showed that such a lymphopenic state can lead to enhanced immune responses, by making room in lymphoid organs for development of T cell responses (2, 14). Furthermore, younger mice, which experience a greater initial lymphopenia relative to older mice, generate a stronger virus-specific immune response (15, 56).

This early depletion may also be important in the context of an infection with a heterologous pathogen, which can cross-react with T cells specific for a previously encountered pathogen. In some cases, memory T cells can cross-react with a heterologous virus, and provide partial protective immunity (24), but in other cases, cross-reactive memory responses may cause a deviation of the immune response, and result in unusual immunopathology (57). Due to the competition between T cells that gives rise to immunodominance, low-affinity cross-reactive memory T cells might prevent the development of more effective high-affinity T cells responding to the immunodominant epitopes that normally dominate during a homologous infection. Therefore, because memory CD8\(^+\) T cells are more susceptible to IFN-dependent early attrition than naive CD8\(^+\) T cells, the loss of cross-reactive memory CD8\(^+\) T cells may reduce their immunodominance and allow for a more diverse immune response. Alternatively, if a cross-reactive memory CD8\(^+\) T cell population was protected from depletion by Ag engagement, it could dominate the immune response and prevent the expansion Ag-specific naive CD8\(^+\) T cells. This phenomenon is indeed predicted by our computer modeling, which shows that if cross-reactive Ag prevents IFN-induced deletion of a memory T cell population, those cross-reactive memory T cells, even those of low affinity, would compete against a more diverse and higher-affinity new T cell response to the newly encountered pathogen (Fig. 6). An early depletion of these cells, even in the presence of TCR engagement with Ag, would allow for a more diverse response to occur. Thus, this early T cell attrition phenomenon that occurs under conditions of virus-induced lymphopenia may not only allow for room in the immune system for a more vigorous T cell expansion but may also allow for a more diverse T cell response originating from naive T cells.

Creating a biological model to study the consequence of the lack of IFN-induced attrition of memory CD8\(^+\) T cells during the early immune response has, thus far, proven difficult, in part because it is difficult to separate the T cell apoptosis-inducing properties of
the IFN response from the myriad of other IFN-induced events that regulate Ag presentation and the immune response. Tg expression of Bel-2 in T cells was not helpful, as it did not render them resistant to the early depletion (R. M. Welch, unpublished observation). We are examining the specific molecular pathways in this T cell apoptosis to see whether there will be a way of blocking the death without affecting other T cell functions. It is noteworthy that there is less attrition of T cells in older mice and that this correlates with reduced magnitudes in the resultant T cell response (15). Perhaps, cross-reactive T cell responses would be more immunodominating in older mice or in a viral infection that induces a weak IFN response.

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Disclosures
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References
Essential role for caspase 8 in T-cell homeostasis and T-cell-mediated immunity. 


