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CD4+ T Cell Help Has an Epitope-Dependent Impact on CD8+ T Cell Memory Inflation during Murine Cytomegalovirus Infection

Christopher M. Snyder, Andrea Loewendorf, Elizabeth L. Bonnett, Michael Croft, Chris A. Benedict, and Ann B. Hill

Murine CMV (MCMV) establishes a systemic, low-level persistent infection resulting in the accumulation of CD8+ T cells specific for a subset of viral epitopes, a process called memory inflation. Although replicating virus is rarely detected in chronically infected C57BL/6 mice, these inflationary cells display a phenotype suggestive of repeated Ag stimulation, and they remain functional. CD4+ T cells have been implicated in maintaining the function and/or number of CD8+ T cells in other chronic infections. Moreover, CD4+ T cells are essential for complete control of MCMV. Thus, we wondered whether CD4+ T cell deficiency would result in impaired MCMV-specific CD8+ T cell responses. Here we show that CD4+ T cell deficiency had an epitope-specific impact on CD8+ T cell memory inflation. Of the three codominant T cell responses during chronic infection, only accumulation of the late-appearing IE3-specific CD8+ T cells was substantially impaired in CD4+ T cell-deficient mice. Moreover, the increased viral activity did not drive increased CD8+ T cell division or substantial dysfunction in any MCMV-specific population that we studied. These data show that CD4+ T cell help is needed for inflation of a response that develops only during chronic infection but is otherwise dispensable for the steady state maintenance and function of MCMV-specific CD8+ T cells. The Journal of Immunology, 2009, 183: 3932–3941.

M urine CMV (MCMV) is a β-herpesvirus that establishes a low-level systemic, persistent infection. A defining feature of the immune response to MCMV is the large numbers of functional, virus-specific CD8+ T cells that accumulate over time, a process termed memory inflation (1–5). In C57BL/6 (B6) mice, inflationary T cells are primarily specific for epitopes from three viral proteins, M38, m139, and IE3. Whereas responses to M38 and m139 are present during the acute infection, IE3-specific cells become readily detectable in the blood only ~3 wk into the infection. At late times postinfection, inflationary T cells specific for all three Ags bear a differentiated effector phenotype (CD27lowIL-7RαlowKLRG-1+) and fail to produce IL-2, indicative of repeated Ag stimulation (4, 5). However, these cells remain functional. We found that most of these inflationary T cells can be stimulated to secrete effector cytokines and were responsive to viral challenge despite their differentiation. Interestingly, the chronic infection did not drive most inflationary T cells to divide during chronic infection. Instead, most differentiated inflationary T cells were short lived in the circulation of chronically infected hosts and had to be constantly replaced by new effector T cells in order for the population to be maintained (5). The phenotype of these inflationary T cells along with the fact that they are constantly being replaced, likely suggest that inflationary T cell populations are maintained in an Ag-dependent manner. Indeed, because MCMV persists below the threshold of detection in C57BL/6 mice, the accumulation, differentiation and maintenance of virus-specific CD8+ T cells is the strongest evidence of persistent viral activity.

However, not all virus-specific T cells accumulate or differentiate over time. CD8+ T cells specific for other viral peptides, such as epitopes in M45 and M57, respond robustly during acute infection but contract afterwards and persist in low, stable numbers. These cells resemble central memory T cells in phenotype (CD27highIL-7RαhighKLRG-1+) and recall capacity. Moreover, the cells appear to be quiescent and maintained by homeostatic division, as if they are ignorant of the persistent viral infection (5). Thus, MCMV infection seems to produce both Ag-dependent (inflationary) and Ag-independent (stable-memory) CD8+ T cells during infection of a single animal.

Much of our understanding of how CD8+ T cells deal with chronic viral infections is derived from work with variants of lymphocytic choriomeningitis virus (LCMV) that produce chronic infections (6–13). These LCMV strains replicate to high titers for prolonged periods of time and drive substantial CD8+ T cell dysfunction. Importantly, CD4+ T cell deficiency both accelerates and exacerbates the CD8+ T cell dysfunction during chronic LCMV infection (9, 10). However, the impact of CD4+ T cell deficiency on CD8+ T cells in other models of chronic viral infection (polyomavirus, murine γ-herpesvirus-68, and HSV), has been somewhat variable (14–23). The combined evidence has led to a model
proposing that the antigenic load and the degree of CD4\textsuperscript{+} T cell impairment are proportional to the extent of CD8\textsuperscript{+} T cell dysfunction (7). According to the model, CD8\textsuperscript{+} T cells remain functional in chronic CMV infection because the viral Ag load is kept very low and/or because CD4\textsuperscript{+} T cell help is available. In consequence, we would expect that increased viral load and/or impaired CD4\textsuperscript{+} T cell help might lead to CD8\textsuperscript{+} T cell dysfunction.

In MCMV infection, CD4\textsuperscript{+} T cells are absolutely required for control of replicating virus in the salivary gland (24) and probably play a direct role in preventing viral recrudescence (25). Thus, CD4\textsuperscript{+} T cell-deficient mice have increased levels of viral replication and also lack CD4\textsuperscript{+} T cell help for CD8\textsuperscript{+} T cells. In wild-type mice, inflationary CD8\textsuperscript{+} T cells appear to be repeatedly stimulated with viral Ag, even though replicating virus is kept below the level of detection. We wondered whether the combination of increased viral replication and an absence of CD4\textsuperscript{+} T cell help would tip the balance and result in progressive CD8\textsuperscript{+} T cell dysfunction during chronic MCMV infection.

Here we show that CD4\textsuperscript{+} T cell deficiency has an epitope-specific impact on memory formation of CD8\textsuperscript{+} T cells during MCMV infection. IE3-specific CD8\textsuperscript{+} T cells, which in wild-type mice appear in significant numbers only during chronic infection, were essentially absent from CD4\textsuperscript{+} T cell-deficient mice. However, despite high titer persistent infection in the salivary glands and the absence of CD4\textsuperscript{+} T cell help, CD8\textsuperscript{+} T cells responses to the two other inflationary epitopes, m139 and M38, developed in near normal numbers and displayed only limited evidence of increased stimulation and dysfunction. Finally, the increased virus replication did not alter the dichotomy between stable and inflationary CD8\textsuperscript{+} T cells.
B6, MHC II
of Ag-specific T cells was measured in the spleens of
ramer staining reagent (Fig. 2, approached our limit of detection with the IE3 peptide-loaded tet-
MHC II numbers; whereas M38-specific T cells were slightly elevated in
icance was measured as in Fig. 1.
ne essential for Ag presentation to CD8 T cells (30). Mice were infected, and the percent of
T cells specific for the indicated peptides was measured in the
over time by intracellular cytokine staining for IFN-γ. For each
sample, responses were compared with unstimulated CD8T cells from
same animal (not shown), and gates were typically set to include
0.1% of these unstimulated CD8 T cells. Each symbol represents an
individual mouse. Significance was measured by Student’s t test. **, p < 0.05; ***, p < 0.001.

A similar impact of CD4 T cell deficiency on both m139- and
IE3-specific T cells in the peripheral blood was seen by tetramer
staining (Fig. 2), excluding the possibility that these responses had
developed, but did not secrete IFN-γ in response to Ag. However,
the epitope-specific impact of CD4 T cell deficiency was slightly
different in the tissues. Mirroring the results in the blood, there
were substantially reduced percentages of IE3-specific T cells in
both the spleen and lungs of CD4 T cell-deficient mice (Fig. 2A),
and M38-specific T cell percentages were normal in both organs
(not shown). However, whereas the percent of m139-specific CD8 T
cells was reduced in the blood of MHC II T mice, they were the
same as in wild-type mice in both lung and spleen (Fig. 2B). In
addition, the absolute number of IE3-specific T cells was substantial-
lly reduced in the spleens of CD4 T-cell-deficient mice, es-
specially MHC II T mice in which the numbers of IE3-specific cells
approached our limit of detection with the IE3 peptide-loaded tet-
ramer staining reagent (Fig. 2, C and D). In both MHC II T mice
and CD4 T mice, m139-specific T cells were present in normal
numbers; whereas M38-specific T cells were slightly elevated in
MHC II T mice (Fig. 2, C and D).

To test whether IE3-specific CD8+ T cells needed CD4+ T cell
help before memory inflation begins (Ref. 4 and Fig. 1), CD4+ T
cells were depleted from B6 mice during the first 3–4 wk of in-
festation. As shown in Fig. 3A, IE3-specific T cells accumulated
normally in the peripheral blood, whereas m139- and M38-specific T
cells were slightly reduced by this treatment. This result con-
trasted with the absent IE3 inflation we had seen in mice geneti-
cally deficient in CD4+ T cells (Figs. 1 and 2) and suggested that
IE3 inflation was not dependent on programming during the acute
infection. However, to validate the comparison between the results
with genetic and Ab depletion, we wanted to confirm that chronic
CD4+ T cell depletion would give the same results that we had
seen with the genetically deficient mice (i.e., reduce IE3-specific T
cell inflation). For this purpose, we used μMT (B cell-deficient)
mice. Because μMT mice cannot reject the depleting Ab, long-
term CD4+ T cell depletion is significantly easier. Indeed, deple-
tion of CD4+ T cells throughout the time course (in this case, 8
wk) eliminated the accumulation of IE3-specific T cells, confirm-
ning our results with genetically deficient mice (Fig. 3B). These
CD4+ T cell-depleted μMT mice had substantial viral replication
in their lungs and spleens as well as salivary glands (101–109 PFU/
organ, not shown), perhaps because the lack of Abs allowed the
virus to spread (31). In addition, depletion of CD4+ T cells only
from wk 3 to wk 8 resulted in reduced IE3-specific T cell accumu-
lation in three of four mice (Fig. 3B). The one mouse in which IE3-
specific T cells inflated normally in this experiment also had incom-
plete CD4+ T cell depletion (Fig. 3B, lower left).

By comparison, noninflammatory T cells behaved like central
memory T cells in CD4 T cell-deficient mice. The absolute
numbers of M45-specific T cells, and a similar population specific
for M57, were both reduced in the spleens of MHC II T mice (Fig.
2C), consistent with previous evidence suggesting that the number
of central memory CD8 T cells can be reduced in CD4 T cell-
deficient mice (32–34). It is still unknown what distinguishes the
epitopes that normally undergo memory inflation from those that
dominate the acute response (4, 27).

FIGURE 2. The reduction of IE3- and m139-specific T cells can be measured by tetramer staining. A, IE3-
specific T cells in the peripheral blood, spleen, and lungs were measured by tetramer staining. Data are
combined from several experiments, and mice were infected for 3–10 mo. B, m139-specific T cells were mea-
sured by tetramer staining as in A. C and D, The number of Ag-specific T cells was measured in the spleens of
B6, MHC II T, and CD4 T mice by tetramer staining. Data are combined from two independent experiments
(MHC II T) or a single experiment (CD4 T). Signif-
ificance was measured as in Fig. 1.
CD40L−/− mice had a significant reduction in the accumulation of IE3-specific CD8+ T cells. In contrast, M38- and m139-specific CD8+ T cells accumulated normally (not shown). There was no evidence of viral replication in the salivary glands of CD40L−/− mice at sacrifice (24 wk postinfection; data not shown), suggesting that continued viral replication was not the sole cause of the deficiency in IE3-specific T cell accumulation.

Other TNF family members have also been shown to impair MCMV-specific T cell inflation. 4-1BB/4-1BBL appears to contribute to memory inflation (I. Humphreys and M. Croft, submitted). MCMV-specific T cell inflation. 4–1BB/4–1BBL appears to contribute to memory inflation (I. Humphreys and M. Croft, submitted). Memory inflation is partially more BrdU than their stable memory (M45- and M57-specific) counterparts. In wild-type mice, inflammatory T cells (M38-, m139-, and IE3-specific) incorporated substantially more BrdU than their stable memory (M45- and M57-specific) counterparts. Thus as early as 2 wk postinfection, when virus was still evident in the salivary glands of wild-type mice, inflammatory T cells were already diverging from the stable memory populations. This observation fits with the previous indication that memory inflation correlates with increased cell division (as assessed by Ki-67 staining) during the first 6 wk of infection (40). However, the elevated viral activity in CD4+ T cell-deficient mice did not translate into increased BrdU incorporation by any of the Ag-specific T cell populations (Fig. 5B). Furthermore, the difference between inflammatory and stable memory populations was preserved: M45- and M57-specific T cells still incorporated low amounts of BrdU in CD4+ T cell-deficient mice when compared with inflammatory T cells in the same animals. In fact, the only statistically significant difference we found indicated that m139-specific T cells divided less over this time period in the absence of CD4+ T cell help. These data show that inflammatory T cells divided more frequently than stable memory T cells at the outset of memory inflation, but that the rate of division was not increased in
and M57-specific CD8+ T cells in MHC II−/− mice displayed slightly elevated BrdU incorporation, suggesting that these cells may be stimulated more in CD4+ T cell-deficient mice, although this did not reach statistical significance (p = 0.1632 for M45-specific T cells; p = 0.2887 for M57-specific T cells). Nevertheless, there was no substantial increase in the BrdU incorporation for any Ag-specific CD8+ T cells in MHC II−/− mice.

Together, these data show for the first time that inflationary cells continue to proliferate after the peak of the primary CD8+ T cell response, whereas stable memory cells do not. Strikingly, in CD4+ T cell-deficient animals, in which the virus persists at high levels in the salivary glands and can be found sporadically in other organs, the kinetics of proliferation of both inflationary and stable memory T cells essentially parallels that found in wild-type animals, where virus is well controlled.

**MCMV-specific T cells display some evidence of increased Ag stimulation in the absence of CD4+ T cells, but do not develop substantial dysfunction**

Because both CD4+ T cell deficiency and chronic viral replication have been shown to promote CD8+ T cell exhaustion, we hypothesized that inflationary CD8+ T cells, which show evidence of repeated Ag encounter even in wild-type animals (4, 5, 40), might develop substantial dysfunction in CD4+ T cell-deficient mice. To investigate this, we examined the phenotype and function of inflationary T cells in wild-type and CD4+ T cell-deficient mice. The vast majority of M38- and m139-specific inflationary T cells in both wild-type and CD4+ T cell-deficient mice expressed low levels of the differentiation markers CD127, CD27, and CD28 and high levels of the inhibitory proteins NKG2A and KLRG-1 (Fig. 6, A and B, and data not shown). These populations were more likely to be extensively differentiated in every MHC II−/− animal, whereas wild-type animals showed a wider distribution of phenotypes (Fig. 6B). However, this modest increase in differentiation of the inflationary populations was only evident in MHC II−/− mice; in CD4−/− mice these populations were indistinguishable from their wild-type counterparts (not shown). For reasons that are unclear, IE3-specific T cells in wild-type mice differ a little in phenotype from the other inflationary epitopes; although they are typically CD27low, they are often heterogeneous for NKG2A expression (Fig. 6, A and B, and Ref. 5). In MHC II−/− mice, these IE3-specific T cells were mostly absent. However, in the occasional MHC II−/− animal which had enough IE3-specific T cells to assess their phenotype, they appeared to have a phenotype similar to that of their wild-type counterparts (Fig. 6A). Finally, M45- and M57-specific T cells that do not inflate and appear phenotypically similar to central memory T cells in B6 animals (CD127hl/CD122hl/b/CD27hl/b/NKG2Atn/KLRG-1tn) showed some evidence of increased differentiation in MHC II−/−, but not CD4−/− mice (Fig. 6, A and B, and data not shown). However, even in MHC II−/− mice, in which replicating virus persists in the salivary gland indefinitely, these stable memory populations did not differentiate extensively to resemble inflationary T cells.

In wild-type animals, neither inflationary nor stable memory CD8+ T cells up-regulate PD-1, which is a hallmark of CD8+ T cell exhaustion (11). There was no evidence of increased PD-1 expression by any MCMV-specific CD8+ T cells in the blood of CD4+ T cell-deficient animals (not shown). However, some Ag-specific cells in the spleen displayed increased PD-1 expression in MHC II−/− mice (Fig. 6, C and D), though the difference was slight and again, not seen in CD4−/− animals (not shown). Thus, the increased amount of viral replication in CD4+ T cell-deficient animals does not substantially alter the phenotype of MCMV-specific CD8+ T cells.
Another hallmark of T cell dysfunction is reduced cytokine expression (7, 10). We saw no evidence of reduced IFN-γ production on a per cell basis from the inflammatory T cells in MHC II−/− mice (Fig. 7A) or CD4−/− mice (not shown). However, these cells are already differentiated and express slightly less IFN-γ per cell than their stable memory counterparts even in B6 animals (Fig. 7). The fraction of M45- and M57-specific T cells that differentiated during chronic infection (in both wild-type and MHC II−/− mice) also produced less IFN-γ per cell than their less differentiated counterparts (Fig. 7A). The example in Fig. 7A shows IFN-γ production by NKG2A-positive and -negative cells, although similar results were found for CD27low and CD127low CD8+ T cells (not shown).

In MHC II−/− mice, the increased differentiation and reduction in IFN-γ production by M45-specific T cells were substantial enough to result in an overall reduction in the amount of IFN-γ produced per cell within this population (Fig. 7B). Although we also saw an increase in differentiated T cells within the M57-specific population, these cells more frequently seemed to retain their ability to secrete high levels of IFN-γ, and the overall amount of IFN-γ produced per cell was not significantly changed.

Most tetramer+, inflammatory CD8+ T cells from chronically infected wild-type mice retained the ability to produce IFN-γ upon stimulation, and this was unchanged in MHC II−/− and CD4−/− mice (Fig. 8A and data not shown). We did find, however, that in MHC II−/− mice there was a small but significant reduction in the percent of M38- and m139-specific IFN-γ producing T cells that also produced TNF-α (Fig. 8D). Nevertheless, even in the absence of CD4+ T cell help, most MCMV-specific T cells retained the capacity to produce both IFN-γ and TNF-α, at least within the timeframe described here (3–5 mo postinfection). The reduced TNF-α expression was not attributable to the MHC II−/− background per se, because no reduction was seen in these mice at day 7 postinfection, the peak of the acute CD8+ T cell response (Fig. 8C). In fact, both M45- and M57-specific T cells exhibited increased TNF-α expression at this early time in MHC II−/− mice. Most inflammatory T cells do not produce IL-2, even in wild-type animals (4), and this was also unchanged in CD4+ T cell-deficient mice (not shown).
Finally, in other model systems, CD4\(^+\) T cell deficiency is often, but not always, associated with poor proliferation in response to Ag challenge (32–34, 43–46). To test whether MCMV-specific CD8\(^+\) T cells primed in CD4\(^+\) T cell-deficient mice also exhibit poor recall activity, we purified CD8\(^+\) T cells from chronically infected MHC II\(^{-/-}\) or wild-type mice, CFSE labeled them, and transferred them separately into naive, congenic recipients. After infection of recipient mice with MCMV, it was apparent that all donor CD8\(^+\) T cells were able to divide extensively, fully diluting CFSE within 6 days of the challenge infection (not shown). However, there were some hints that CD8\(^+\) T cells from MHC II\(^{-/-}\) mice expanded less than their wild-type counterparts. Thus, to directly compare the proliferative capacity of each population, purified CD8\(^+\) T cells from wild-type mice (CD45.1\(^+\)Thy1.2\(^+\)) or MHC II\(^{-/-}\) mice (CD45.1\(^+\)Thy1.2\(^+\)) were cotransferred into naive, Thy1.1 (CD45.1\(^+\)Thy1.2\(^+\)) recipients. Recipients were then challenged and MCMV-specific donor cells (Thy1.2\(^+\)CD8\(^+\) T cells; Fig. 10A) were analyzed 7 days later. Although Ag-specific CD8\(^+\) T cells from both donor populations underwent clonal expansion, the wild-type CD8\(^+\) T cells consistently expanded to a greater degree (Fig. 10, B–D). When compared directly within each mouse, the wild-type CD8\(^+\) T cells expanded between 2× and 8× better than CD8\(^+\) T cells with the same specificity from MHC II\(^{-/-}\) mice (Fig. 10D). However, when we analyzed BrdU uptake by Ag-specific T cells over the final 24 h (days 6–7), the CD8\(^+\) T cells from MHC II\(^{-/-}\) mice were largely unimpaired in BrdU incorporation (Fig. 10E). Thus, although the CD8\(^+\) T cells from MHC II\(^{-/-}\) mice failed to accumulate to the same degree as their wild-type counterparts, this may not be exclusively due to a lack of T cell division. Rather, it is possible that CD8\(^+\) T cells from MHC II\(^{-/-}\) mice also survive more poorly than wild-type CD8\(^+\) T cells, as has been suggested elsewhere (43).

Together, our data show that whereas one inflationary population (IE3 specific) failed to accumulate, the M38- and m139-specific T cell populations inflated normally and did not become markedly exhausted in CD4\(^+\) T cell-deficient mice, despite the elevated levels of viral replication in these animals. These cells were fully differentiated in phenotype and were not dividing extensively, consistent with the notion of a linkage between exhaustion and Ag-driven proliferation (47). However, CD4\(^+\) T cell deficiency did affect the ability of MCMV-specific CD8\(^+\) T cells to expand in response to acute viral challenge, similar to many experiments with acute, cleared infections (32, 33, 43–46). This impairment was seen in both the inflationary and stable memory populations and is consistent with the notion of altered programming during their initial priming, rather than being related to dysfunction induced by chronic infection. However, this reduced recall capacity failed to substantially affect the steady-state maintenance of inflationary CD8\(^+\) T cells that developed during chronic MCMV infection of CD4\(^+\) T cell-deficient mice.

**Discussion**

Although it is clear that CD4\(^+\) T cell help is important for the generation or maintenance of memory CD8\(^+\) T cells in acute, cleared infections, the role of CD4\(^+\) T cells in persistent viral infections has varied with the infectious model. Here we investigated the impact of CD4\(^+\) T cell deficiency on the chronic CD8\(^+\) T cell response to MCMV in B6 mice. Because CD4\(^+\) T cell deficiency results in high-titer MCMV persistence in the salivary gland, we were looking at the combined impact of an increased antigenic burden and the absence of CD4\(^+\) T cell help. We expected that this would result in an increased activation state and perhaps inflation of the normally stable memory cells and a shift toward exhaustion in the inflationary cells. Instead, we found that,
although recall responses were diminished, the pattern and function of both stable and inflammatory memory responses during chronic infection were remarkably similar to those of the wild type. The single exception was the absence of a detectable inflammatory response to the normally codominant IE3 epitope.

It is unclear at this time why IE3-specific CD8<sup>+</sup> T cells differ from m139- and M38-specific CD8<sup>+</sup> T cells in their CD4<sup>+</sup> T cell dependency, although it seems likely that the cause is related to the different kinetics of the responses (Fig. 1 and Ref. 4). Because IE3-specific CD8<sup>+</sup> T cells are not prominent early in infection, it is tempting to speculate that they must be primed at late times, after the majority of the virus has been controlled. Indeed, we have shown that IE3-specific T cells (as well as m139- and M38-specific T cells) can be primed at late times and during memory inflation in B6 mice (Ref. 5 and data not shown). Although inflammatory signals may be able to license dendritic cells during acute infection, thus priming m139- and M38-specific T cells, CD4<sup>+</sup> T cells may be required to license dendritic cells after viral replication has been largely controlled. Analogous results were obtained after infection with polyomavirus (14). In these experiments, CD4<sup>+</sup> T cell deficiency resulted in reduced priming of new virus-specific T cells during chronic infection and thus fewer virus-specific T cells at steady state. The reduction of IE3-specific CD8<sup>+</sup> T cells in CD40L<sup>−/−</sup> mice is also consistent with the hypothesis that priming of IE3-specific T cells is impaired in CD4<sup>+</sup> T cell-deficient mice. CD40 is important for dendritic cell licensing (36, 37) and could be vital for licensing dendritic cells during chronic MCMV infection.

It is also possible that the survival or maintenance of IE3-specific CD8<sup>+</sup> T cells is dependent on CD4<sup>+</sup> T cell help. During chronic infection with virulent strains of LCMV, T cells specific for some epitopes are deleted, which is considered to be an extreme stage of exhaustion (6, 7, 9, 10, 13). Hence, one could imagine that IE3-specific T cells are deleted in CD4<sup>+</sup> T cell-deficient mice due to severe exhaustion. However, the fact that the other inflammatory populations showed no sign of exhaustion makes this seem less likely.

Exhaustion of CD8<sup>+</sup> T cells in chronic LCMV infection is characterized by a progressive loss of the ability of cells to produce IL-2, followed by TNF-α and ultimately IFN-γ. During MCMV infection in wild-type animals, inflammatory CD8<sup>+</sup> T cells fail to make IL-2, but produce both TNF-α and IFN-γ upon peptide stimulation (4, 5). Although CD4<sup>+</sup> T cell-deficient mice have increased viral replication and no CD4<sup>+</sup> T cell help, the vast majority of M38- and m139-specific CD8<sup>+</sup> T cells continued to produce both TNF-α and IFN-γ, suggesting that they were not becoming progressively dysfunctional. In fact, a reduced recall capacity was the only obvious functional defect in the MCMV-specific CD8<sup>+</sup> T cells that we measured. However, this is a well-established effect donor populations between the adoptive transfer and day 7 postchallenge. Statistical significance was determined as in Fig. 1. D, Wild-type T cells expand more than CD8<sup>+</sup> T cells from MHC II<sup>−/−</sup> mice in each animal. The ratio of wild-type to MHC II<sup>−/−</sup> Ag-specific T cells in the spleen 7 days after viral challenge was normalized to the ratio of cells that were injected (based on tetramer stains of the donor populations on the day of injection). Shown is the normalized data plotted on a log scale. The dotted line indicates a value of 1, which would result from equal expansion of the CD8<sup>+</sup> T cells from wild-type and MHC II<sup>−/−</sup> mice. The fact that all values are above this line indicates that the wild-type donor CD8<sup>+</sup> T cells expanded more than the CD8<sup>+</sup> T cells from MHC II<sup>−/−</sup> mice in each animal. E, CD8<sup>+</sup> T cells from wild-type and MHC II<sup>−/−</sup> mice incorporate similar amounts of BrdU after viral challenge. Mice were pulsed with BrdU 24 h before sacrifice. Shown is the percent of tetramer<sup>+</sup> T cells in the spleen that incorporated BrdU. Statistical significance was calculated as in Fig. 1.
of CD4+ T cell deficiency on CD8+ T cell memory in many models of acute cleared infections and is linked to programming during acute infection rather than exhaustion (32, 33, 43–46).

CD4+ T cell deficiency exacerbates and accelerates T cell exhaustion in the chronic LCMV model of infection (9, 10). However, similar to our results with MCMV, CD4+ T cell deficiency has a much more limited impact on CD8+ T cell function during chronic infections of polyomavirus, HSV, or murine γ-herpesvirus-68 (14–19, 22, 23). Wherry and Ahmed (48) have suggested that repeated antigen stimulation is the main determinant of T cell exhaustion and that different viral loads during chronic infection primarily account for the differences seen with these different models. Thus, perhaps some differences between infection models can be ascribed to the absolute amount of virus Ag. Even in CD4+ T cell-deficient animals, the MCMV burden is markedly lower than in chronic LCMV infection (Ref. 24 and Fig. 5C). MCMV replicates to higher titers in the absence of effective NK cell control (such as in BALB/c mice or A/J157 virus in B6 mice), but even in these cases CD4+ T cell-deficient animals maintain functional antiviral CD8+ T cells (Fig. 9 and Ref. 24). Nevertheless, these manipulations still do not result in the level of virus burden that is seen in chronic LCMV infection, particularly in central organs, and the concept of Ag load as a determinant of T cell exhaustion remains attractive.

MCMV is used as a model for human CMV infection. In general, human CMV-specific CD8+ T cells also remain functional throughout the chronic, lifelong infection. However, in transplant recipients there are periods of viremia, and these have been shown to correlate with CD8+ T cell dysfunction (49–52). These periods of viremia likely represent vastly more systemic antigenic load than is found during MCMV infection, even in CD4+ T cell-deficient mice, which is consistent with antigenic load as a major determinant of T cell dysfunction.

Another question driving our experiments was whether ongoing, high-level viral replication would disrupt the normal dichotomy between stable and inflationary cells. The determinants of inflationary antiviral CD8+ T cells and effector function of the other CD8+ T cells populations were not substantially impaired despite the ongoing high level of viral replication and lack of CD4+ T cell help. Thus, at the level of viral persistence seen in our model, CD4+ T cell help is not a dominant factor in the maintenance of functional MCMV-specific CD8+ T cells.

Disclosures

The authors have no financial conflict of interest.

References


