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The β-herpesvirus CMV induces a substantial and progressive expansion of virus-specific memory CD8 T cells, which protect the host against viral reactivation from latency. In this paper, we report that this expansion, or “inflation,” of memory T cells is amplified dramatically during mouse CMV infection of IL-10 knockout (IL-10−/−) mice. T cells from IL-10−/− mice were oligoclonal, exhibited a highly activated phenotype, expressed antiviral cytokines, and degranulated in response to cognate Ag encounter ex vivo. Moreover, latent viral load was reduced in IL-10−/− mice. Importantly, these results were recapitulated by IL-10R blockade during chronic/latent infection of wild-type mice. These data demonstrate that regulatory immune mechanisms can influence CMV-specific T cell memory and suggest a possible rationale for the acquisition of functional IL-10 orthologs by herpesviruses. The Journal of Immunology, 2010, 185: 3583–3592.
Materials and Methods

**Mice and ethics**

IL-10−/− mice were purchased from The Jackson Laboratory (Bar Harbor, ME) and bred in-house; wild-type C57BL/6 mice were purchased from Harlan U.K. (Blackthorn, U.K.). All experiments were conducted according to the U.K. Home Office guidelines. Specifically, these experiments were performed under a Home Office project license (granted to I.R.H.) in the Home Office-designated facility at Heath Park, Cardiff University (Cardiff, U.K.).

**Virus, infections, and treatments**

MCMV Smith strain (American Type Culture Collection, Manassas, VA) was prepared in BALB/c salivary glands and titered on 3T3 cells as described previously (38). Mice were infected i.p. with 5 × 10⁶ PFU MCMV. In some experiments, wild-type mice were injected on the days stated in the figure legends with 250 μg of either rat IgG (Chemogenic International, Temecula, CA) or anti-IL-10Rα (clone 1B1.3A.5a; BioXCell, West Lebanon, NH).

**Intracellular cytokine staining and flow cytometry**

To evaluate MCMV-specific CD8 T cell responses, splenocytes and lung leukocytes were isolated from infected mice and incubated at 5 × 10⁶ cells/ml for 5 h at 37˚C with 2 μg/ml MCMV-derived peptides (GenScript, Piscataway, NJ) in the presence of either 0.7 μg/ml monensin and anti–CD107a–FITC (both BD Pharmingen, San Diego, CA; IFN-γ and TNF-α detection) or 2 μg/ml brefeldin A (Sigma-Aldrich, St. Louis, MO; IFN-γ, TNF, and IL-2 detection). The optimal length peptides used in this study were as follows: 1) SSPPMFRVP (origin, M38; restriction, H-2Kb); 2) NHLYETPISATAMVI (origin, M25); 3) TRPYRYPRVCDASLS (origin, M142) (39). To identify the optimal peptide concentrations, we titrated a range of peptide concentrations from 10⁻⁵ to 10⁻¹ μg/ml, and found that 2 μg/ml was optimal for inducing CD107a degranulation in our system (data not shown). To detect CD107a degranulation or cytokine production in medium controls was subtracted from peptide-induced mobilization or expression, respectively.

To identify MCMV-specific CD4 T cells, splenocytes and lung leukocytes were incubated for 5–16 h at 37˚C with 2 μg/ml brefeldin A (Sigma-Aldrich) and 3 μg/ml MCMV-derived peptides (GenScript) restricted by MHC class II as follows: 1) GYLYIYTPASNDFL (origin, m99); 2) NHLYETPISATAMVI (origin, M25); 3) TRPYRYPRVCDASLS (origin, m139; restriction, H-2Kd; and 4) RALEYKYNL (origin, immediate-early 3 (IE3); restriction, H-2Kb) (20). Cells were then washed and incubated with Fc block (eBioscience, San Diego, CA). After incubation with peptide or medium alone as a control, cells were surface stained with anti–CD4-FITC, anti–CD8-PE, anti–CD44-PE-Cy5, anti–CD62L-PerCP-Cy5.5, anti–Gr1-FITC (BD Pharmingen), anti–MHC class II-PE-Cy5 (eBioscience), anti–F4/80-Pacific blue (BioLegend, San Diego, CA), anti–CD11c-PE-Cy7 (BD Pharmingen), anti–CD11b-PE-Cy7 prior to permeabilization and intracellular staining with anti–IFN-γ-FITC, or anti–CD11b-PE-Cy7. To identify T helper type 1 (Th1)- and Th2-type CD4 T cells, we used human Th1 and Th2-activation peptide pools (Genscript), which were used in a similar manner to identify MCMV-specific CD8 T cells. A minimum of 30,000 events was acquired in each case, and data were analyzed using FlowJo software version 8.5.3 (Tree Star, Ashland, OR).

In all experiments, total numbers of different cell populations were calculated by multiplying the total number of viable splenic or pulmonary leukocytes (assayed by trypan blue exclusion) by percent positive cells, as detected by flow cytometry. Total numbers of virus-specific T cells were calculated by: total T cell number (CD4 or CD8) × percent peptide-specific IFN-γ+ cells (calculated as described above). Total numbers of virus-specific TNF or CD107a+ T cells were calculated similarly.

**TCR clonotyping**

Clonotypic analysis of cognate Ag-specific CD8 T cell populations was conducted as described previously with minor modifications (40, 41). Briefly, tetramer-labeled CD8 T cells (median, 3200; range, 808–5000) were sorted viable into 1.5-ml microtubes containing 100 μl RNA later (Applied Biosystems, Foster City, CA), and unbiased amplification of all expressed TCRB gene products was conducted using a template-switch anchored RT-PCR with a 3˚ TCRB C region primer (5'-TGGCTCAAA-CAAGGAGACCT-3'). Amplicons were subcloned, sampled, sequenced, and analyzed as described previously (42). The International ImMunoGeneTics Information System nomenclature is used throughout this report (43).

**Viral genome detection**

Genomic DNA was isolated from spleen and lung tissue (Qiagen, Valencia, CA), and MCMV glycoprotein B (gB) was then assayed by quantitative PCR using a Mini Optipon (Bio-Rad, Hercules, CA) and Platinum SYBR green mastermix reagent (Invitrogen). One hundred-nanogram aliquots of DNA were used as templates for each reaction. The primer sequences used for detection of β-actin were 5'-GATGCTACGCGAGATTTCC-3' and 5'-GGCTATGCTTCCTCTAC-3'; primers used for detection of gB were 5'-GAAGATCCGGATGCTTCTCAG-3' and 5'-AATCGGCTCAACATCTTGGTCG-3'. Genome copy numbers were calculated using a standard curve generated with the pARK25 MCMV plasmid (a gift from A. Redwood, University of Western Australia, Perth, Western Australia, Australia) with the limit of detection being 10 copies.

**gB and IL-10 gene expression**

IL-10 and gB were assayed by quantitative RT-PCR using a Mini Optipon and Platinum SYBR green mastermix reagent (Invitrogen). For IL-10 expression analysis, lung and spleen cells from mock or MCMV-infected mice were frozen on dry ice in TRZol reagent (Invitrogen). Thawed samples were homogenized, and total cellular DNA was extracted and quantified. DNase-treated RNA was then used to synthesize cDNA with a TaqMan reverse transcription kit (Applied Biosystems). The primer sequences used for detection of IL-10 were 5'-ACGATGCCGCAAGAACAACT-3' and 5'-CGCCATTGGCTTCATCA-3'. For gB expression analysis, RNA was isolated from lung and spleen tissue (Qiagen); cDNA was then synthesized as described above, and gB and actin were measured using the primers detailed above.

**Virus reactivation assay**

Virus-infected organs were divided into three parts and placed in separate wells in a 6-well plate containing 5 ml (spleen) or 3 ml (lung) D10 medium. Tissue was gently minced with the end of a 2-ml syringe, and in the case of lung pieces, an additional 2 ml D10 was then added. Tissue pieces were then cultured for 5 wk; 4 ml supernatant was collected weekly and replaced with 5 ml fresh D10. Sonicated supernatant was then assayed for infectious virus by plaque assay as described previously (38).

**Results**

**IL-10 limits memory CD8 T cell expansion during MCMV infection**

In our C57BL/6 mouse model of MCMV infection, productive replication (data not shown) and late viral gene expression (Supplemental Fig. 1A) were undetectable in the spleen and lung by 60 d postinfection. In contrast, viral DNA was present in both organs, and virus could reactivate ex vivo in cultures of spleen and lung isolated as late as 110 d postinfection (Supplemental Fig. 1B). We then asked if IL-10 limits memory CD8 T cell expansion during MCMV infection (44). Briefly, tetramer-labeled CD8 T cells (median, 3200; range, 808–5000) were sorted viable into 1.5-ml microtubes containing 100 μl RNA later (Applied Biosystems, Foster City, CA), and unbiased amplification of all expressed TCRB gene products was conducted using a template-switch anchored RT-PCR with a 3˚ TCRB C region primer (5'-TGGCTCAAA-CAAGGAGACCT-3'). Amplicons were subcloned, sampled, sequenced, and analyzed as described previously (42). The International ImMunoGeneTics Information System nomenclature is used throughout this report (43).
Flow cytometric analyses revealed IL-10 protein production by splenic IFN-γ/IL-17 MCMV-specific CD4 T cells after peptide stimulation directly ex vivo (Fig. 1B). The frequency of IL-10–producing cells within MCMV-specific CD4 T cell populations was substantially higher than that observed in IFN-γ+CD4 T cells (3.7–8.3%, mean = 6.3 versus 0.08–0.3%, mean = 0.19%; p < 0.01). Spleenic B cells (CD19+) and monocytes/macrophages (CD11b+) derived from mice 90 d postinfection were also capable of producing IL-10 following ex vivo nonspecific stimulation; in the case of CD11b+ cells, but not CD19+ cells, this MCMV-associated IL-10 production was significantly increased compared with cells derived from naive mice (Fig. 1C).

We hypothesized that IL-10 acts to inhibit systemic and mucosal antiviral T cell responses during MCMV infection. To test this, we measured the accumulation of both “stable” memory CD8 T cells specific for a peptide derived from the M45 protein that is immunodominant during acute infection and “inflationary” memory CD8 T cells specific for peptides derived from the M38, m139, and IE3 proteins in both wild-type C57BL/6 and IL-10−/− mice. Expression of these viral proteins in infected cells is thought to occur during

![Graphs and images](http://www.jimmunol.org/content/images/content/1087446f.jpg)
productive replication rather than viral latency (20, 44). During acute infection, there were comparable numbers of MCMV-specific CD8 T cells in the lungs and spleens of wild-type and IL-10−/− mice (Fig. 1D); furthermore, as infection progressed, the numbers of M45-specific memory CD8 T cells remained low in the absence of IL-10 (Fig. 1D). Consistent with previously published data (20), high frequencies of CD8 T cells specific for M38, m139, and IE3 were observed in wild-type mice at day 90 postinfection; strikingly, however, the corresponding frequencies at the same time point were even higher in IL-10−/− mice (Fig. 1D). The accumulation of IE3-specific CD8 T cells was most notable, representing ~15% of all CD8 T cells in IL-10−/− mice at day 90 postinfection (Fig. 1E) and reaching up to 30% in some individual mice (data not shown).

**IL-10 limits the oligoclonal expansion of IE3-specific CD8 T cells**

To investigate further the profound expansion of IE3-specific CD8 T cells, we undertook a comprehensive and unbiased analysis of TCR gene usage within the cognate populations isolated directly ex vivo from both wild-type and IL-10−/− mice (Fig. 2 and, for complete data set, Supplemental Fig. 2). In both sets of mice at day 90 postinfection, the IE3-specific TCR repertoires were oligoclonal and highly skewed toward the usage of only a few dominant clonotypes; this is consistent with previous studies of HCMV-specific CD8 T cell populations (42). Moreover, a diverse array of clonotypic structures was apparent in both wild-type and IL-10−/− mice, although with a strong bias toward TRBV16 usage (Fig. 2, Supplemental Fig. 2). Similar patterns were observed as late as day 282 postinfection, at which time IE3-specific CD8 T cells dominated the response in IL-10−/− mice (Supplemental Figs. 3–5). In addition, a substantial degree of differentially encoded interindividual TCR sharing was apparent (Fig. 2, Supplemental Figs. 2, 4, and 5); this is consistent with recent studies demonstrating the role of convergent recombination in the generation of “public” clonotypes, which can affect the outcome of infection in certain circumstances (45, 46). Overall, these data suggest that the enhanced inflation of IE3-specific CD8 T cells in IL-10−/− mice is due to the expansion of clonotypes selected within the memory pool rather than the recruitment of greater numbers of cognate clonotypes from the naive pool (47).

**Enhanced MCMV-specific CD4 T cell and professional APC accumulation in IL-10−/− mice during MCMV infection**

CD4 T cells promote MCMV-specific memory CD8 T cell expansion (48, 49). We therefore measured the accumulation of virus-specific memory CD4 T cell populations in wild-type and IL-10−/− mice, investigating previously described distinct CD4 T cell populations with specificities for four different viral proteins (m09, M25, m139, and m142) that are associated with active viral replication (39). Interestingly, we found that enhanced CD8 T cell accumulation in IL-10−/− mice at day 90 postinfection was accompanied by a large increase in the number of IFN-γ–expressing CD4 T cells in the spleen (Fig. 3A, 3B) and lung (Fig. 3C, 3D) detected following ex vivo stimulation with MCMV-derived MHC class II-restricted peptides (Fig. 3A, 3C) or PMA and ionomycin (Fig. 3B, 3D). IL-10 can inhibit CD4 T cells by suppressing the function of professional APCs (26, 27) and MCMV-induced IL-10 inhibits MHC class II expression (50). In accordance, we observed more splenic DCs (Fig. 3E, 3F), splenic inflammatory monocytes (Fig. 3G), and pulmonary macrophages (Fig. 3H) in IL-10−/− mice compared with wild-type mice. In contrast, B cell accumulation was comparable in spleens and lungs of wild-type and IL-10−/− mice (data not shown).

**IL-10 inhibition of CD8 T cell memory occurs during acute and chronic/latent MCMV infection**

MCMV-specific memory CD8 T cell populations are derived both from cells primed during acute infection and from more recent thymic emigrants (22). Given that IL-10 is expressed during acute MCMV infection (39, 51–53) and suppresses CD4 (52) and, in some models, CD8 T cell responses (53), at this time, we next examined the time point at which IL-10 exerts its inhibitory effect.

**FIGURE 2.** Oligoclonal expansion of IE3-specific CD8 T cells during MCMV infection. TRCB3 CDR3 amino acid sequences, TRCBR and TRBJ usage, and the relative frequencies of individual clonotypes within viable CD3+ CD8+IE3 tetramer+ cell populations isolated by flow cytometric sorting from wt (A) and IL-10−/− (B) mice at day 90 post-MCMV infection. Three representative mice are shown per group; colored boxes represent shared clonotypes that were detected in other individual mice. The full data set is shown in Supplemental Fig. 2; analyses from later time points are shown in Supplemental Fig. 4 (day 142 postinfection) and Supplemental Fig. 5 (day 282 postinfection).
Next, we inhibited IL-10R signaling from day 60 postinfection. Importantly, CD8 T cell populations specific for M38, m139, and IE3 were all substantially increased (3- to 4-fold) following late IL-10R blockade (Fig. 4E). In the case of IE3-specific CD8 T cells, this increased accumulation was more dramatic than that observed after early IL-10R blockade (Fig. 4A). Thus, IL-10 primarily limits memory T cell accumulation during the chronic/latent phase of infection.

CD27 expression is downregulated by MCMV-specific CD8 T cells from IL-10−/− mice

In contrast to the phenotypic heterogeneity of the total CD8 T cell population (Fig. 5A), inflammatory MCMV-specific memory CD8 T cells from wild-type mice were CD44hiCD62Llo (Fig. 5A), consistent with an effector memory status. Furthermore, these highly differentiated cells expressed high levels of KLRG-1 and low levels of CD27 (Fig. 5A). Memory CD8 T cells specific for MCMV from IL-10−/− mice were also CD44hiCD62Llo (Fig. 5A). Notably, however, the frequency of KLRG-1+CD27+ MCMV-specific CD8 T cells was even higher in IL-10−/− mice (Fig. 5A, 5B), and downregulation of CD27 was more pronounced (Fig. 5A, 5C). In addition, receptors for the homeostatic cytokines IL-7 and IL-15 were not upregulated by CD8 T cells from IL-10−/− mice (data not shown). Collectively, these data suggest that IL-10 does not inhibit memory CD8 T cells via the regulation of inhibitory or cytokine receptor expression; furthermore, it seems that the enhanced expansion of inflammatory MCMV-specific CD8 T cells in the absence of IL-10 is associated with more advanced differentiation.

IL-10 restricts the accumulation of functional MCMV-specific CD8 T cells

We next hypothesized that IL-10 limits the accumulation of functional antiviral memory CD8 T cells. Hence, we examined cytokine production by MCMV-specific CD8 T cells. A low proportion (6–10%) of wild-type MCMV-specific IFN-γ+ CD8 T cells coexpressed IL-2 following ex vivo peptide stimulation, and this was further reduced (2–4%) in IL-10−/− mice (Fig. 6A). In contrast, IL-10 deficiency increased both the proportion (Fig. 6A) and the total number (Fig. 6B) of MCMV-specific IFN-γ+ CD8 T cells capable of coexpressing the antiviral cytokine TNF. We also quantified recent degranulation based on cell surface CD107a mobilization and observed greater numbers of CD107a+ MCMV-specific CD8 T cells in the spleens and lungs of IL-10−/− mice compared with wild-type mice after ex vivo peptide stimulation (Fig. 6C). Importantly, IL-10R blockade in wild-type mice from
FIGURE 5. IL-10−/− MCMV-specific CD8 T cells are highly differentiated. MCMV-specific CD8 T cells from spleens of wt and IL-10−/− mice were detected with cognate tetramers and coexpression of CD27, KLRG-1, CD44, and CD62L, was examined. A, Coexpression of CD27 and KLRG-1 (left panels), and CD62L and CD44 (right panels), by CD3+CD8+ tetramer+ cells (colored dots) and total CD3+CD8+ cells (black/gray) from spleens of wt (left panels in each set) and IL-10−/− (right panels in each set) mice. B and C, The frequencies of KLRG-1−CD27+ CD3+CD8+ tetramer+ cells (B) and the MFI of CD27 expression by CD3+CD8+ tetramer+ cells (C) were assessed. In IL-10−/− mice, the %KLRG-1−CD27+ cells in the total CD3+CD8+ population was not significantly increased (wt = 23.5, IL-10−/− = 24.1; \(p = 0.91\)) nor was the CD27 MFI (wt = 2564.75, IL-10−/− = 1979.5, \(p = 0.223\)).

Discussion

In this paper, we report that IL-10 suppresses CMV-specific memory T cell inflation. The key findings of this study were that 1) chronic/latent MCMV infection was associated with IL-10 production by macrophages and virus-specific IFN-γ-expressing CD4 T cells; 2) mice deficient in IL-10 exhibited a profound increase in MCMV-specific CD8 T cell accumulation and antiviral cytokine production; 3) IL-10 suppressed the oligoclonal expansion of memory CD8 T cells; 4) MCMV viral genome load was reduced in IL-10−/− mice; and 5) the enhanced memory CD8 T cell expansion and reduced viral load observed in IL-10−/− mice was recapitulated by delayed IL-10R blockade in wild-type mice. Collectively, these data demonstrate that IL-10 restricts the accumulation of functional memory MCMV-specific T cells during MCMV infection.

In vivo, MCMV infection leads to chronic activation of APCs (54) and the generation of highly differentiated, activated memory CD8 T cells (22, 24); these observations suggest that, after the resolution of acute infection, mice harbor nonreplicating, reactivating and, possibly, low levels of chronically replicating CMV that drives the immune activation (55–57). In the current study, 3.7–8.3% of splenic IFN-γ+ MCMV-specific memory CD4 T cells from MCMV-infected mice expressed IL-10. The production of IL-10 by Th1-like cells is triggered by chronic stimulation in parasitic infections (58, 59), and prolonged stimulation of HCMV-specific CD4 T cells in vitro induces IL-10/IFN-γ coexpression (60). Similarly, we observed that MCMV infection led to an increased frequency of IL-10−/− expressing CD11b+ cells. Although we cannot exclude the possibility that MCMV may encode a protein(s) that selectively induces IL-10 expression by these cell subsets, our data imply that IL-10 expression by MCMV-specific CD4 T cells and monocytes/macrophages is a consequence of chronic activation, which, in this model, is likely to be provided by persistent viral reactivation from latency and, possibly, undetectable chronic replication.

B cells are a significant source of IL-10 during acute systemic MCMV infection (53). Although chronic/latent infection did not increase the proportion of IL-10−/−producing CD19+ cells in the spleen as compared with naive mice, we cannot exclude the possible importance of this cell subset. Intracellular cytokine staining is a relatively insensitive technique and may therefore underestimate the frequency of IL-10−/−producing CD19+ cells. Furthermore, given that ~50% of splenocytes were CD19+ at day 90 postinfection (I. Humphreys, unpublished observations), even the small percentage of CD19+ IL-10 producers suggested by our data could have a significant impact on antiviral immunity.

Interestingly, although IL-10 expression was detected in the lungs of mice at day 90 postinfection, this was not increased compared with naive controls (data not shown). Whether or not this reflects the small numbers of virus-specific CD4 T cells in the lungs of chronic/latently infected mice in our model, or the presence of IL-10−/−producing interstitial macrophages (61) in both uninfected and infected mice, is not clear.
The amplification of CD8 T cell inflation in IL-10−/− mice was striking and maintained 10 mo postinfection without causing overt signs of disease (weight loss and cachexia) or pathology (M. Jones and I. Humphreys, unpublished observations). In some models, IL-10 directly inhibits CD8 T cells (62, 63), whereas in other situations, IL-10 actually promotes primary (64) and memory CD8 T cell responses (64, 65). In our system, inflationary CD8 T cells expressed low levels of IL-10R (data not shown), thereby suggesting that these cells might be refractory to direct IL-10–mediated effects. Interestingly, enhanced CD8 T cell responses in IL-10−/− mice were accompanied by increased numbers of CD4 T cells and APCs. Inflationary CD8 T cell responses are partially dependent on CD4 T cells (48, 49), thereby implying that elevated CD4 T cell help in IL-10−/− mice promoted CD8 T cell expansion. Interestingly, IE3-specific CD8 T cells, which are more sensitive to CD4 depletion than other memory CD8 T cells (49), were preferentially expanded in IL-10−/− mice. Collectively, these data suggest that IL-10 limits memory CD8 T cell expansion via the abrogation of effective CD4 T cell help.

Expansion of CD8 T cell numbers was not observed 7 d postinfection, as reported previously (52). Primary MCMV-specific CD8 T cell responses occur independently of CD4 T cell help (48, 49),
suggesting that IL-10–mediated inhibition of CD4 T cells only limits memory CD8 cells. Interestingly, however, B cell–derived IL-10 inhibits primary CD8 T cell responses following s.c. infection (53). Whether or not these differences reflect the different mouse strains or infection routes used in this study, variation in how peptide–specific CD8 T cells were enumerated (percentages versus total numbers of splenic CD8 T cells) or the fact that we housed our IL-10−/− mice in specific pathogen-free conditions (and subsequently did not observe colitis in aged naive mice or mice infected with MCMV for 14 mo; data not shown) is unclear.

The increased expansion of CD8 T cells in IL-10−/− mice was not due to altered expression of cytokine receptors (CD122 and CD127) or the absence of inhibitory molecules (KLRG-1). However, it is known that virus-specific CD8 T cells downregulate CD27 during latent MCMV infection (24, 66), and we found that the expression of this surface marker was further reduced in IL-10−/− mice. One possible functional consequence of CD27 downregulation in this study was reduced IL-2 production in response to Ag stimulation; this is consistent with previous studies linking cellular differentiation to the loss of IL-2 expression (67, 68) and suggests that IL-10 restricts MCMV-specific cytotoxic T cell responses.

In chronic lymphocytic choriomeningitis virus infection, dysregulated TNF production by CD8 T cells is rescued in IL-10−/− mice (33). However, in MCMV infection, virus-specific CD8 T cells sustain proinflammatory cytokine expression during latency (22). Indeed, 60–70% of virus-specific IFN-γ–producing CD8 T cells in wild-type mice coexpressed TNF. Therefore, enhanced TNF production by IL-10−/− CD8 T cells probably reflects increased activation rather than reversal of defective function. Furthermore, CD8 T cell functionality was not enhanced on a per cell basis following IL-10R blockade in wild-type mice (data not shown), highlighting that the qualitative restriction of CD8 T cell function by IL-10 was comparatively small as compared with the large effect on T cell numbers. Collectively, these data suggest that IL-10 predominately regulates inflammatory CD8 T cell responses by inhibiting cellular accumulation at sites of viral infection.

Importantly, MCMV burden was reduced in IL-10−/− mice and wild-type mice treated with an IL-10R blocking Ab. Expanded CD8 T cells in both situations were reactive to several viral proteins expressed during active replication, thereby implying that these cells do not directly recognize latently infected cells. Of particular interest was the expansion of IE3-specific CD8 T cells. IE genes are expressed during the early stages of viral replication, and IE3 is a checkpoint gene involved in the maintenance of latency (72). Patrolling IE–specific CD8 T cells may detect early reactivation events (73). It is therefore possible that the increased frequency of Ag recognition by IE3-specific cells may contribute to the more dramatic effect of IL-10 on this population. In support of this hypothesis, latent MCMV infection of BALB/c mice induces the inflation of IE1–specific CD8 T cells (19, 21) that terminate viral reactivation in vivo (74). Alternatively, however, it is conceivable that enhanced memory T cell responses in the absence of IL-10R signaling may inhibit low levels of undetectable chronic replication, which, in turn, restricts the establishment of latent infection in the spleen and lung.

The exact mechanism by which expanded CD8 T cells reduced viral load following IL-10 gene deletion or IL-10R blockade is also unclear. Both approaches led to enhanced numbers of CD8 T cells in the spleen and lung that could degranulate efficiently; in conjunction with the reduced levels of CD27 expression, this implies an enhanced capability to kill cells harboring reactivating and/or replicating virus. Indeed, elevated TNF secretion by CD8 T cells in the absence of IL-10R signaling may promote splicing of IE3 transcripts and subsequent viral reactivation (75). In this situation, which could be implied by the preferential expansion of IE3–specific CD8 T cells in IL-10−/− mice, increased numbers of cytotoxic T cells would detect and kill cells harboring this reactivating virus. Alternatively, increased accumulation of T cells expressing IFN-γ and TNF might directly inhibit late gene expression during viral reactivation and/or replication (76–78) and may also activate secondary antiviral mechanisms mediated by other cell types, such as macrophages and NK cells (79–81).

The factors that determine whether a MCMV–specific CD8 T cell population inflates are currently unknown. However, the observation that inflammatory but not stable memory CD8 T cells are enhanced in IL-10−/− mice implies that IL-10 is not a determining factor in this process. Rather, IL-10 appears to limit the expansion of memory CD8 T cells that are already dividing during chronic/latent infection.

The data presented in this paper provide evidence that, as previously suggested (37, 82), IL-10 orthologs expressed by herpesviruses including HCMV (34) and EBV (83) may function to preserve latent viral load within the host. It should be noted, however, that MCMV does not encode an obvious IL-10 ortholog, and the kinetics and locality of expression of host and viral IL-10 proteins may differ in vivo. Furthermore, HCMV (84) and EBV (85) IL-10 orthologs promote B cell growth and differentiation,
whereas in our model, we observed no effect of murine IL-10 numbers of CD4 T cells in IL-10−/− mice may compensate for the absence of direct IL-10 signaling in B cells, we cannot exclude the possibility that mammalian and viral IL-10 proteins could function differently in vivo. Irrespective, our data clearly highlight the previously unappreciated role for mammalian IL-10 in the regulation of memory T cell inflation in vivo and suggest that this immune regulatory pathway could be manipulated to promote the accumulation of functional antiviral memory T cells.

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Disclosures
The authors have no financial conflicts of interest.

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