Published in final edited form as:
Nat Immunol. 2004 September ; 5(9): 927–933. doi:10.1038/ni1105.

CD4+ T cells are required for the maintenance, not programming, of memory CD8+ T cells after acute infection

Joseph C Sun, **Matthew A Williams**, and **Michael J Bevan**
Department of Immunology and the Howard Hughes Medical Institute, University of Washington, Seattle, Washington 98195, USA

Abstract

Immunization in the absence of $CD4^+$ T cell help results in defective $CD8^+$ T cell memory, deficient recall responses and diminished protective immunity. Here we investigated at what stage during the immune response to pathogen $CD4^+$ T cells are essential in the promotion of functional $CD8^+$ T cell memory. Memory $CD8^+$ T cell numbers decreased gradually in the absence of $CD4^+$ T cells despite the presence of similar numbers of memory cell precursors at the peak of the effector phase. Adoptive transfer of effector or memory $CD8^+$ T cells into wild-type or $CD4^+$ T cell–deficient mice demonstrated that the presence of $CD4^+$ T cells was important only after, not during, the early $CD8^+$ T cell programming phase. In the absence of $CD4^+$ T cells, memory $CD8^+$ T cells became functionally impaired and decreased in quantity over time. We conclude that in the context of an acute infection, $CD4^+$ T cells are required only during the maintenance phase of long-lived memory $CD8^+$ T cells.

The rapid and more efficacious response of memory T cells after the second encounter with a pathogen constitutes a hallmark trait of adaptive immunity. The ‘canonical’ $CD8^+$ T cell response to an acute infection consists of three well defined phases: the proliferation of naive cells to produce large numbers of effector cells; the contraction of these effector populations into memory cells once antigen is cleared; and the long-term maintenance of these memory cells. A brief encounter with antigen, as short as 24 h, can initiate ‘programmed differentiation’ in activated $CD8^+$ T cells that leads to many rounds of antigen-independent division and the acquisition of effector function[1–4]. Effector cell populations at the peak of a CD8 response are also programmed to contract during their priming[5]. Thus, during an immune response to infection, intrinsic and external signals ‘dictate’ or program the complete $CD8^+$ T cell response[6,7].

Although $CD4^+$ T cells are not required for primary $CD8^+$ T cell responses against pathogens, memory $CD8^+$ T cell numbers and secondary responses to bacterial or viral challenge are decreased over time in a $CD4^+$ T cell–deficient animal[8–11]. In those studies[8–11], it was not apparent when the $CD4^+$ T cell help was required to enhance the survival of the antigen-specific memory $CD8^+$ T cells. It has been speculated that $CD4^+$ T cells interacting with antigen-presenting cells also engage in crosstalk with $CD8^+$ T cells during the proliferation or programming phase, delivering the necessary ‘instructive’ and/or survival signals for the generation of a fully functional memory $CD8^+$ T cell pool[12–14]. It has also been suggested that

Correspondence should be addressed to M.J.B. (mbevan@u.washington.edu).

Note: Supplementary information is available on the Nature Immunology website.

COMPETING INTERESTS STATEMENT

The authors declare that they have no competing financial interests.

Published online at http://www.nature.com/natureimmunology/

signals given during the contraction or maintenance phases regulate homeostasis of the memory population[14–16]. Because activated $CD8^+$ T cell populations undergo a program that dictates their subsequent contraction and eventual memory cell frequencies, we sought to understand at what phase of the immune response to pathogen $CD4^+$ T cells are crucial in determining memory $CD8^+$ T cell numbers and functionality. Are naive $CD8^+$ T cells programmed early during the expansion phase by $CD4^+$ T cell help to become productive memory cells? Or do $CD4^+$ T cells provide an important contribution in the maintenance of memory $CD8^+$ T cell numbers later during the memory phase?

Expression of the interleukin 7 receptor α-chain (IL-7Rα) chain at the peak of the $CD8^+$ T cell primary response marks those effector cells that will differentiate into memory cells[17,18]. Here we found that effector $CD8^+$ T cells expressing IL-7Rα were present in equal numbers after infection in wild-type and MHC class II–deficient mice, providing evidence that the formation of memory cell precursors does not require $CD4^+$ T cell help. We then 'dissected apart' the stages of the $CD8^+$ T cell response to determine precisely when $CD4^+$ T cell help is important for sustained CD8 memory of previously encountered antigen. By generating effector or memory $CD8^+$ T cells in either wild-type or MHC class II–deficient mice and adoptively transferring them into normal or $CD4^+$ T cell–deficient secondary recipients, we found that $CD4^+$ T cell help was not crucial in the generation of functional CD8 memory during the primary expansion phase (days 0–8 after infection), when naive cells are programmed and differentiate to become potent effectors. In the context of an acute infection, $CD4^+$ T cells are crucial to the health and numbers of memory $CD8^+$ T cells only during the memory maintenance phase.

Results

Memory decrease in $CD4^+$ T cell–deficient mice

Endogenous memory $CD8^+$ T cells in $CD4^+$ T cell–deficient but not wild-type mice decrease in number over time[8]. We recapitulated that finding here, tracking adoptively transferred $CD8^+$ T cells specific for lymphocytic choriomeningitis virus (LCMV) glycoprotein amino acids 33–41 (GP(33–41); P14 T cell receptor–transgenic, Thy-1.1^+) in wild-type or MHC class II–deficient mice after infection with recombinant *Listeria monocytogenes* expressing the LCMV GP(33–41) epitope (rLmGP) or after infection with LCMV. We tracked numbers of P14 cells by staining for Thy-1.1, MHC class I tetramer and intracellular interferon-γ (IFN-γ). After either bacterial (Fig. 1a) or viral (Fig. 1b) infection, P14 cell numbers expanded similarly in both hosts to generate large numbers of effectors, which then contracted. Memory $CD8^+$ T cell numbers in the spleen remained almost constant in wild-type mice beyond day 21 after infection, but there was a continuous decrease in memory P14 cell numbers in MHC class II–deficient mice, resulting in a reduction of 85–90% in absolute numbers at 100 d or more after infection, compared with that of wild-type hosts. This decrease in memory cell numbers found in the spleens of MHC class II–deficient mice also occurred in the liver and lymph nodes (Supplementary Fig. 1 online). This concordance between different tissues is not unexpected, given that memory $CD8^+$ T cells in central lymphoid organs and in nonlymphoid tissues are in rapid equilibrium[19]. Thus, our results do not indicate a specific loss in either 'effector' or 'central' memory pools in $CD4^+$ T cell–deficient mice. We analyzed the surface phenotype and cytokine production of effector and memory cells in wild-type and MHC class II–deficient mice. Although effector cells in the two environments did not differ, we noted profound differences in CD62L and CD122 (but not CD44) expression on memory cells in wild-type versus MHC class II–deficient mice. We also found an overall reduction in IFN-γ and IL-2 production by the memory cell populations in the MHC class II–deficient mice (Supplementary Fig. 2 online). These findings suggest a diminished functional capacity of memory cells generated in MHC class II–deficient hosts. We obtained similar results in studies in which we

adoptively transferred naive ovalbumin (OVA)–specific, OT-1 T cell receptor–transgenic $CD8^+$ T cells into wild-type or MHC class II–deficient mice, followed by infection with recombinant *L. monocytogenes* expressing chicken OVA (data not shown). In all experiments, the infection was cleared in both wild-type and MHC class II–deficient mice 8 d after infection and was not detected at any subsequent memory time points (data not shown). These findings led us to explore at what stage during the response to an acute infection $CD4^+$ T cells is crucial in sustaining memory $CD8^+$ T cell numbers and functionality.

Generation of memory cell precursors is CD4 independent

The subset of effector $CD8^+$ T cells that express IL-7Rα (CD127) 8 d after LCMV infection is predisposed to become long-lived memory cells[17]. Over 90% of naive T cell receptor–transgenic $CD8^+$ T cells have high expression of IL-7Rα (Fig. 2a). Because we found reduced memory cell numbers in a $CD4^+$ T cell–deficient environment, we compared IL-7Rα expression on effector $CD8^+$ T cells 8 d after LCMV infection in wild-type and MHC class II–deficient mice, with the idea that memory cell precursor generation might depend on the presence of $CD4^+$ T cells. Equal percentages (about 15–20%) of IL-7Rα^{hi} effector $CD8^+$ T cells were present in the two sets of mice at day 8 after infection (Fig. 2b). The similar IL-7Rα^{hi} memory cell precursor numbers at day 8, however, did not ‘translate’ into equal memory cell numbers at later stages in the different host environments (Fig. 1). We compared surface IL-7Rα expression on memory cells from the two environments at day 100 after infection and found that although almost all of the antigen-specific memory $CD8^+$ T cells in wild-type mice expressed IL-7Rα, most of the remaining memory cells in MHC class II–deficient mice did not (Fig. 2b). Therefore, the loss of memory $CD8^+$ T cells in MHC class II–deficient mice correlates with diminished IL-7Rα expression at later time points and not with expression of this receptor at the effector stage.

$CD4^+$ T cells are important after programming

We sought to more precisely pinpoint the timing of the $CD4^+$ T cell function in determining the fate of the memory $CD8^+$ T cell population. We primed P14 cells in wild-type mice with LCMV and, on day 8 after infection, transferred equal numbers of purified effector cells into wild-type or MHC class II–deficient recipients and tracked donor $CD8^+$ T cell numbers by Thy-1.1 staining in the spleen at 3, 30 and 60 d after transfer (Fig. 3). We analyzed samples at day 3 in this as well as subsequent experiments to ensure that the ‘take’ (engraftment) of donor cells was equivalent in all mice. As expected, effector $CD8^+$ T cell numbers contracted after transfer into wild-type or MHC class II–deficient recipients. However, effector $CD8^+$ T cell numbers underwent a substantial continuing contraction in MHC class II–deficient secondary recipients, resulting in 75–80% lower absolute memory cell numbers by day 60 after transfer compared with those of wild-type recipients (Fig. 3a,c). Similarly, effector $CD8^+$ T cells generated in MHC class II–deficient primary hosts experienced a decrease in total numbers of greater than 80% after transfer into MHC class II–deficient compared with those of wild-type secondary recipients 60 d later (Fig. 3b,d).

Effector cell populations generated in wild-type or MHC class II–deficient primary hosts showed a similar contraction and maintenance of memory cell numbers when transferred into wild-type secondary recipients (Fig. 3a,b). Between days 30 and 60 after transfer, the absolute number of memory cells from both sources remained relatively stable in wild-type secondary hosts. Comparison of the two effector $CD8^+$ T cell populations transferred into MHC class II–deficient recipients showed a similar continuous decrease in memory cell numbers at the later time points. Irrespective of the host environment during programming, both donor cell populations decreased in the MHC class II–deficient recipients. Experiments involving the adoptive transfer of OVA-specific effector $CD8^+$ T cells primed with the recombinant *L. monocytogenes* expressing chicken OVA gave similar results (data not shown). These findings

demonstrate that the presence of $CD4^+$ T cells is important only after the period of expansion of the $CD8^+$ T cell response.

Transferred effectors become functionally impaired

We investigated whether the remaining memory $CD8^+$ T cells in MHC class II–deficient recipients were functionally impaired compared with memory cells in wild-type recipients. We purified effector $CD8^+$ T cells generated in wild-type or MHC class II–deficient primary hosts and transferred them into wild-type or MHC class II–deficient secondary recipients. Using intracellular cytokine staining, we measured the ability of the cells to rapidly produce both IFN-γ and IL-2 in response to peptide stimulation at 3 and 60 d after transfer. By comparing the mean fluorescence intensity of IFN-γ staining, we noted a decreased ability of the transferred memory $CD8^+$ T cells in MHC class II–deficient host mice to produce IFN-γ at 60 d after transfer (Fig. 4a,b). This was accompanied by a 65% decrease in the fraction of memory cells that were capable of IL-2 production (Fig. 4c,d). In accordance with published findings[9], memory $CD8^+$ T cells maintained in $CD4^+$ T cell–deficient environments are characterized by this overall decrease in cellular fitness.

To assess the ability of effector $CD8^+$ T cells from wild-type mice to confer protective immunity 60 d after transfer to wild-type or MHC class II–deficient secondary recipients, we challenged recipient mice with a high dose of rLmGP and 3 d later measured bacterial clearance in spleen (Fig. 5a) and liver (Fig. 5b). As a control, we infected naive mice that did not receive transferred effector cells; these mice were unable to control growth of this high dose of listeria. Wild-type mice that had received effector cells 60 d before showed a high degree of protection against the challenge. In contrast, MHC class II–deficient recipients of effector cells showed only minimal protection against the challenge. These results provide further evidence that the maintenance of protective $CD8^+$ T cell memory depends on the presence of $CD4^+$ T cells.

$CD4^+$ T cells are required during maintenance of memory

We adoptively transferred memory $CD8^+$ T cells from either wild-type or MHC class II–deficient primary hosts 35 d after infection into wild-type or MHC class II–deficient secondary recipients and tracked memory cell numbers by Thy-1.1 staining. In this set of experiments, we transferred memory cells that had undergone identical expansion and contraction phases into different secondary environments to study memory maintenance. We compared total donor $CD8^+$ T cell numbers in the spleen at 3, 30 and 60 d after transfer (Fig. 6). Memory $CD8^+$ T cells from wild-type primary hosts decreased in number in MHC class II–deficient secondary recipients compared with wild-type secondary recipients, resulting in almost 90% lower absolute numbers by day 60 after transfer (Fig. 6a,c). Similarly, memory $CD8^+$ T cells generated in MHC class II–deficient primary hosts underwent a loss of 75–80% when transferred into MHC class II–deficient compared with that of wild-type secondary recipients (Fig. 6b,d). The unexpected increase in memory cell numbers in the wild-type secondary recipients over the course of the study can be attributed to the young age of the mice (4–5 weeks at the time of transfer), as total spleen cellularity more than doubled by day 60 after transfer in both wild-type and MHC class II–deficient mice (data not shown). These findings demonstrate that $CD4^+$ T cells are crucial for sustaining the memory $CD8^+$ T cell pool only after this stable, long-lived population is generated. In addition, the results in Figure 6b suggest that decreasing memory $CD8^+$ T cells from MHC class II–deficient primary hosts at day 35 after infection can be rescued by transfer to a wild-type environment containing $CD4^+$ T cells.

Discussion

Even though a primary $CD8^+$ T cell response may seem to be independent of $CD4^+$ T cells, there is a requirement for CD4 help for the subsequent generation of stable, protective CD8

memory[8–11,20,21]. An important issue arising from those studies[8–11,20,21] is when during an immune response $CD4^+$ T cells provide signals that promote the survival of long-lived memory $CD8^+$ T cells. Is $CD4^+$ T cell help needed early during the primary response in a 'covert' way that is revealed only at later stages? Or are $CD4^+$ T cells required at later stages to sustain memory $CD8^+$ T cells? In this study, we have shown that during an acute infection, the generation of memory $CD8^+$ T cell precursors is $CD4^+$ T cell independent and have provided evidence that the presence of $CD4^+$ T cells during memory maintenance is crucial to memory $CD8^+$ T cell health and survival.

In the cytotoxic T lymphocyte (CTL) response to noninfectious antigens (such as minor histocompatibility antigens, tumor antigens or protein antigen), which are not nonspecifically inflammatory, $CD4^+$ T cell recognition of antigen is important in activating or 'licensing' the antigen-presenting cell such that it can promote a $CD8^+$ T cell response[22–24]. Whether all the functions provided by $CD4^+$ T cells are mediated via the antigen-presenting cell or via direct CD4-CD8 T cell interactions is not known. Some studies have suggested that in response to noninflammatory antigen, the function of the $CD4^+$ T cell becomes apparent only after the primary effector response; without $CD4^+$ T cell help during the first 3–4 d, effector $CD8^+$ T cells may be generated, but they are unable to mount productive recall responses *in vitro*[11,20]. In contrast, other studies have shown that after *in vivo* priming with noninflammatory antigens, $CD4^+$ T cell help is essential for the stimulation of a measurable primary $CD8^+$ T cell response[25–27]. The discrepancy between these results probably hinges on the different amounts of endogenous 'danger' signals produced by the immunization. Further work is needed to elucidate the contribution of $CD4^+$ T cells to CD8 memory maintenance after priming with noninfectious antigens.

In the CTL response to virulent pathogens, such as *L. monocytogenes* and LCMV, the situation is very different. In contrast to the suggestion that direct crosstalk between $CD4^+$ and $CD8^+$ T cells via CD40 ligand and CD40 is necessary for the promotion of robust memory generation with noninflammatory antigens[20], direct CD40–CD40 ligand interaction between $CD4^+$ and $CD8^+$ T cells is not involved during bacterial or viral infections[26,28]. In addition, our results presented here show that in these infections, $CD4^+$ T cells do little to promote or program the $CD8^+$ T cell response during the early phase. Effector or memory $CD8^+$ T cells taken from control or $CD4^+$ T cell–deficient mice acted similarly when adoptively transferred to new hosts. Thus, $CD8^+$ T cells generated in a wild-type mouse decrease in number in a $CD4^+$ T cell–deficient secondary host, and $CD8^+$ T cells generated in a $CD4^+$ T cell–deficient mouse recover and are maintained when transferred to a $CD4^+$ T cell–sufficient secondary host. In all scenarios, the environment of the secondary host completely dictates the health and size of the memory $CD8^+$ T cell pool.

Our findings indicate that the $CD4^+$ T cell function in the maintenance of $CD8^+$ T cell memory is not antigen specific simply because antigen is no longer present. Furthermore, because we purified $CD8^+$ T cells before adoptive transfer into secondary recipients, antigen-specific memory $CD4^+$ T cells or other adaptive immune cells were not present. This raises the question of mechanism. What are the noncognate signals $CD4^+$ T cells provide that allow for memory $CD8^+$ T cell health and survival? It is likely that cytokines, which promote survival and basal homeostatic proliferation of memory $CD8^+$ T cells, are involved. $CD4^+$ T cells either directly provide these factors to memory $CD8^+$ T cells or act on an intermediate cell that provides memory $CD8^+$ T cells with such signals.

Studies have focused on the functions of IL-7 and IL-15 in maintaining CD8 memory[16,29,30]. Because IL-7 is also required for the survival and basal homeostatic proliferation of naive $CD8^+$ T cells[31], and normal numbers of these cells are found in MHC class II–deficient mice, it is unlikely that defects in basal IL-7 production account for diminished memory $CD8^+$ T cell

numbers. This is in line with our findings that IL-7 mRNA expression in the spleen and lymph nodes of wild-type and MHC class II–deficient mice (measured by real-time PCR) were similar (data not shown). Furthermore, we found that naive $CD8^+$ T cells adoptively transferred into MHC class II–deficient recipients did not decrease in number and were maintained for several months, as with wild-type recipients (data not shown). Studies have shown that IL-7Rα^{hi} effector $CD8^+$ T cells are predisposed to become long-lived memory cells, and as in our study here, those studies found that most memory $CD8^+$ T cells express IL-7Rα in wild-type mice[17,18]. Here, although MHC class II–deficient mice and wild-type mice had similar numbers of effector cells that were IL-7Rα^{hi}, IL-7Rα expression was not sustained in the absence of $CD4^+$ T cells. How IL-7Rα expression is lost over time in the absence of $CD4^+$ T cells and whether diminished IL-7Rα expression on memory cells is directly responsible for their lack of maintenance remain to be determined.

IL-15 has been linked to the generation and maintenance of memory $CD8^+$ T cells[32–39]. *Il15*$^{-/-}$ and *Il15ra*$^{-/-}$ mice have deficiencies in memory $CD8^+$ T cell numbers. The kinetics of the $CD8^+$ T cell response after infection in these mutant mice seem very similar to those of the memory $CD8^+$ T cell decrease we noted in MHC class II–deficient mice. Expression of the high affinity IL-15Rα on the $CD8^+$ T cells themselves is dispensable for CD8 memory, although binding of IL-15 to the low-affinity receptor composed of the IL-15Rβ and the γ_c subunits on memory $CD8^+$ T cells is required for their maintenance[40,41]. Expression of the high-affinity IL-15Rα on other (non-CD8) bone marrow–derived cells is essential for the basal homeostatic proliferation of memory $CD8^+$ T cells[40,42], possibly by binding IL-15 and '*trans*-presenting' it to $CD8^+$ T cells[43]. We found no difference in IL-15 mRNA expression in the spleens and lymph nodes of control mice and mice lacking $CD4^+$ T cells (data not shown). Whether $CD4^+$ T cells are responsible for producing a factor for memory $CD8^+$ T cell maintenance or for acting on other cells to provide the cytokine signals required for sustaining memory $CD8^+$ T cells remains to be determined. Elucidation of the function of $CD4^+$ T cells during the memory maintenance phase of the $CD8^+$ T cell response will aid in the design of vaccines, which rely heavily on robust secondary $CD8^+$ T cell responses to pathogens, and will also provide insight into the loss of immune function after depletion of $CD4^+$ T cells in certain disease states.

Methods

Mice

Age-matched C57BL/6 and MHC class II–deficient (C57BL/6-$A^b\beta^{-/-}$) mice were purchased from Taconic. A colony of Thy-1.1^+ P14 transgenic mice was maintained at our specific pathogen–free animal facility at the University of Washington (Seattle, Washington). Experiments began when mice were approximately 6–12 weeks of age; all experiments were done according to institutional ethical guidelines.

Adoptive transfer of cells

Single-cell suspensions were made from spleen of P14 mice, and 5×10^4 gp33-specific cells were injected intravenously into C57BL/6 or MHC class II–deficient mice before infection. At day 8 or day 35 after infection, $CD8^+$ T cells from spleens of mice were purified with a $CD8^+$ T cell Isolation kit (Miltenyi Biotec) followed by AutoMACS magnetic bead separation. Approximately 5×10^6 to 7×10^6 P14 effector cells and 1×10^6 to 2×10^6 P14 memory cells were injected intravenously into recipient mice. At 3, 30 or 60 d after transfer, total numbers of P14 cells in the spleens of recipient mice were determined by Thy-1.1 staining.

Bacterial and viral infections

The rLmGP used was provided by H. Shen (University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania)[44]. Frozen stocks of the rLmGP were grown in brain-heart infusion broth. Bacteria culture samples were grown to mid-log phase, measured by optical density (absorbance at 600 nm) and diluted in PBS for injection. All mice were infected by intravenous injection of the tail vein with a priming dose of 2,000 rLmGP and challenge doses equivalent to 1×10^5 to 2×10^5. Injected bacteria numbers were determined by spreading of bacterial samples on brain-heart infusion plates followed by incubation overnight at 37 °C. LCMV Armstrong 53b was grown on BHK cells and was titered on Vero cells. Mice were infected intraperitoneally with 2×10^5 plaque-forming units of virus.

Determination of colony-forming units

For determination of the colony-forming units per spleen or liver in the infected mouse, the entire organ was dissociated in PBS with 0.1% Nonidet-P40 (Sigma). Tenfold serial dilutions of spleen or liver suspensions were made in PBS plus 0.1% Nonidet-P40, and each dilution was spread on brain-heart infusion plates. Plates were counted after incubation overnight at 37 °C, and total colony-forming units per organ were determined.

MHC class I tetramers and antibodies used for cell staining

H-2D^b tetramers bound to LCMV GP(33–41) were generated as described[45,46]. Splenocytes (1×10^6) were stained with D^{b}GP33 tetramer or antibody to Thy-1.1 (anti-Thy-1.1; BD PharMingen) along with anti-CD8 (PharMingen), anti-CD44 (PharMingen), anti-CD62L (PharMingen), anti-CD122 (PharMingen), or anti-CD127 (eBioscience). Flow cytometry was done on a FACSCalibur and data were analyzed with CELLQuest software (Becton Dickinson).

Intracellular cytokine staining

Intracellular cytokine staining was done with a kit (PharMingen), following the manufacturer's instructions. In 96-well plates, 2×10^6 splenocytes per well were stimulated for 5 h with media alone or with 10 nM GP(33–41) peptide in the presence of 1 μg/ml of Brefeldin A. Cells were then washed, stained with anti-CD8, resuspended in permeabilization-fixation buffer and stained with anti-IFN-γ and anti-IL-2 (PharMingen). Labeled cells were washed in permeabilization buffer, resuspended in fixation buffer and analyzed on a FACSCalibur.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Supported by the National Institutes of Health (AI19335, AI056809 and CA09537) and the Howard Hughes Medical Institute.

References

1. Mercado R, et al. Early programming of T cell populations responding to bacterial infection. J Immunol 2000;165:6833–6839. [PubMed: 11120806]
2. Kaech SM, Ahmed R. Memory CD8$^+$ T cell differentiation: initial antigen encounter triggers a developmental program in naive cells. Nat Immunol 2001;2:415–422. [PubMed: 11323695]
3. van Stipdonk MJ, Lemmens EE, Schoenberger SP. Naive CTLs require a single brief period of antigenic stimulation for clonal expansion and differentiation. Nat Immunol 2001;2:423–429. [PubMed: 11323696]

4. van Stipdonk MJ, et al. Dynamic programming of $CD8^+$ T lymphocyte responses. Nat Immunol 2003;4:361–365. [PubMed: 12640451]
5. Badovinac VP, Porter BB, Harty JT. Programmed contraction of $CD8^+$ T cells after infection. Nat Immunol 2002;3:619–626. [PubMed: 12055624]
6. Bevan MJ, Fink PJ. The CD8 response on autopilot. Nat Immunol 2001;2:381–382. [PubMed: 11323687]
7. Masopust D, Kaech SM, Wherry EJ, Ahmed R. The role of programming in memory T-cell development. Curr Opin Immunol 2004;16:217–225. [PubMed: 15023416]
8. Sun JC, Bevan MJ. Defective CD8 T cell memory following acute infection without CD4 T cell help. Science 2003;300:339–342. [PubMed: 12690202]
9. Shedlock DJ, Shen H. Requirement for CD4 T cell help in generating functional CD8 T cell memory. Science 2003;300:337–339. [PubMed: 12690201]
10. Belz GT, Wodarz D, Diaz G, Nowak MA, Doherty PC. Compromised influenza virus-specific $CD8^+$-T-cell memory in $CD4^+$-T-cell-deficient mice. J Virol 2002;76:12388–12393. [PubMed: 12414983]
11. Janssen EM, et al. $CD4^+$ T cells are required for secondary expansion and memory in $CD8^+$ T lymphocytes. Nature 2003;421:852–856. [PubMed: 12594515]
12. Lanzavecchia A. Immunology. Licence to kill. Nature 1998;393:413–414. [PubMed: 9623994]
13. Tanchot C, Rocha B. CD8 and B cell memory: same strategy, same signals. Nat Immunol 2003;4:431–432. [PubMed: 12719734]
14. Marrack P, Kappler J. Control of T cell viability. Annu Rev Immunol 2004;22:765–787. [PubMed: 15032596]
15. Sprent J, Surh CD. T cell memory. Annu Rev Immunol 2002;20:551–579. [PubMed: 11861612]
16. Schluns KS, Lefrancois L. Cytokine control of memory T-cell development and survival. Nat Rev Immunol 2003;3:269–279. [PubMed: 12669018]
17. Kaech SM, et al. Selective expression of the interleukin 7 receptor identifies effector CD8 T cells that give rise to long-lived memory cells. Nat Immunol 2003;4:1191–1198. [PubMed: 14625547]
18. Huster KM, et al. Selective expression of IL-7 receptor on memory T cells identifies early CD40L-dependent generation of distinct $CD8^+$ memory T cell subsets. Proc Natl Acad Sci USA 2004;101:5610–5615. [PubMed: 15044705]
19. Klonowski KD, et al. Dynamics of blood-borne CD8 memory T cell migration *in vivo*. Immunity 2004;20:551–562. [PubMed: 15142524]
20. Bourgeois C, Rocha B, Tanchot C. A role for CD40 expression on $CD8^+$ T cells in the generation of $CD8^+$ T cell memory. Science 2002;297:2060–2063. [PubMed: 12242444]
21. Khanolkar A, Fuller MJ, Zajac AJ. CD4 T cell-dependent CD8 T cell maturation. J Immunol 2004;172:2834–2844. [PubMed: 14978084]
22. Bennett SR, et al. Help for cytotoxic-T-cell responses is mediated by CD40 signalling. Nature 1998;393:478–480. [PubMed: 9624004]
23. Ridge JP, Di Rosa F, Matzinger P. A conditioned dendritic cell can be a temporal bridge between a $CD4^+$ T-helper and a T-killer cell. Nature 1998;393:474–478. [PubMed: 9624003]
24. Schoenberger SP, Toes RE, van der Voort EI, Offringa R, Melief CJ. T-cell help for cytotoxic T lymphocytes is mediated by CD40-CD40L interactions. Nature 1998;393:480–483. [PubMed: 9624005]
25. Wang JC, Livingstone AM. Cutting edge: $CD4^+$ T cell help can be essential for primary $CD8^+$ T cell responses *in vivo*. J Immunol 2003;171:6339–6343. [PubMed: 14662830]
26. Sun JC, Bevan MJ. Cutting edge: long-lived CD8 memory and protective immunity in the absence of CD40 expression on CD8 T cells. J Immunol 2004;172:3385–3389. [PubMed: 15004136]
27. Le Bon A, et al. Cross-priming of $CD8^+$ T cells stimulated by virus-induced type I interferon. Nat Immunol 2003;4:1009–1015. [PubMed: 14502286]
28. Lee BO, Hartson L, Randall TD. CD40-deficient, influenza-specific CD8 memory T cells develop and function normally in a CD40-sufficient environment. J Exp Med 2003;198:1759–1764. [PubMed: 14657225]

29. Jameson SC. Maintaining the norm: T-cell homeostasis. Nat Rev Immunol 2002;2:547–556. [PubMed: 12154374]
30. Prlic M, Lefrancois L, Jameson SC. Multiple choices: regulation of memory CD8 T cell generation and homeostasis by interleukin (IL)-7 and IL-15. J Exp Med 2002;195:F49–F52. [PubMed: 12070294]
31. Schluns KS, Kieper WC, Jameson SC, Lefrancois L. Interleukin-7 mediates the homeostasis of naive and memory CD8 T cells *in vivo*. Nat Immunol 2000;1:426–432. [PubMed: 11062503]
32. Goldrath AW, et al. Cytokine requirements for acute and basal homeostatic proliferation of naive and memory $CD8^+$ T cells. J Exp Med 2002;195:1515–1522. [PubMed: 12070279]
33. Tan JT, et al. Interleukin (IL)-15 and IL-7 jointly regulate homeostatic proliferation of memory phenotype $CD8^+$ cells but are not required for memory phenotype $CD4^+$ cells. J Exp Med 2002;195:1523–1532. [PubMed: 12070280]
34. Kieper WC, et al. Overexpression of interleukin (IL)-7 leads to IL-15-independent generation of memory phenotype $CD8^+$ T cells. J Exp Med 2002;195:1533–1539. [PubMed: 12070281]
35. Becker TC, et al. Interleukin 15 is required for proliferative renewal of virus-specific memory CD8 T cells. J Exp Med 2002;195:1541–1548. [PubMed: 12070282]
36. Schluns KS, Williams K, Ma A, Zheng XX, Lefrancois L. Cutting edge: requirement for IL-15 in the generation of primary and memory antigen-specific CD8 T cells. J Immunol 2002;168:4827–4831. [PubMed: 11994430]
37. Judge AD, Zhang X, Fujii H, Surh CD, Sprent J. Interleukin 15 controls both proliferation and survival of a subset of memory-phenotype $CD8^+$ T cells. J Exp Med 2002;196:935–946. [PubMed: 12370255]
38. Lodolce JP, et al. IL-15 receptor maintains lymphoid homeostasis by supporting lymphocyte homing and proliferation. Immunity 1998;9:669–676. [PubMed: 9846488]
39. Ku CC, Murakami M, Sakamoto A, Kappler J, Marrack P. Control of homeostasis of $CD8^+$ memory T cells by opposing cytokines. Science 2000;288:675–678. [PubMed: 10784451]
40. Schluns KS, Klonowski KD, Lefrancois L. Transregulation of memory CD8 T-cell proliferation by IL-15Rα^+ bone marrow-derived cells. Blood 2004;103:988–994. [PubMed: 14512307]
41. Burkett PR, et al. IL-15Rα expression on $CD8^+$ T cells is dispensable for T cell memory. Proc Natl Acad Sci USA 2003;100:4724–4729. [PubMed: 12671073]
42. Schluns KS, et al. Distinct cell types control lymphoid subset development by means of IL-15 and IL-15 receptorα expression. Proc Natl Acad Sci USA 2004;101:5616–5621. [PubMed: 15060278]
43. Dubois S, Mariner J, Waldmann TA, Tagaya Y. IL-15Rα recycles and presents IL-15 *in trans* to neighboring cells. Immunity 2002;17:53–547.
44. Shen H, et al. Recombinant *Listeria monocytogenes* as a live vaccine vehicle for the induction of protective anti-viral cell-mediated immunity. Proc Natl Acad Sci USA 1995;92:3987–3991. [PubMed: 7732018]
45. Altman JD, et al. Phenotypic analysis of antigen-specific T lymphocytes. Science 1996;274:94–96. [PubMed: 8810254]
46. Murali-Krishna K, et al. Counting antigen-specific CD8 T cells: a reevaluation of bystander activation during viral infection. Immunity 1998;8:177–187. [PubMed: 9491999]

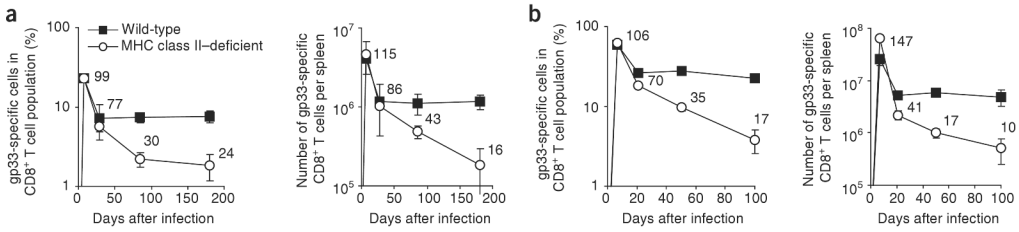

Figure 1.

Gradual decrease in memory $CD8^+$ T cell numbers in MHC class II–deficient versus wild-type mice. Wild-type and MHC class II–deficient mice containing 5×10^4 P14 cells (Thy-1.1) were immunized with rLmGP (**a**) or LCMV (**b**), and $CD8^+$ T cell responses specific for GP(33–41) (gp33) were measured by MHC class I tetramer staining as well as by intracellular IFN-γ staining. The percentage of antigen-specific cells in the total $CD8^+$ T cell population (left) and absolute number of antigen-specific cells in whole splenocyte population (right) were determined at various time points after infection (horizontal axes). Numbers above symbols indicate the percentage of $CD8^+$ T cells from MHC class II–deficient mice versus wild-type mice. Data are presented as the mean ± standard deviation of three to five mice per group at each time point and are representative of three independent studies.

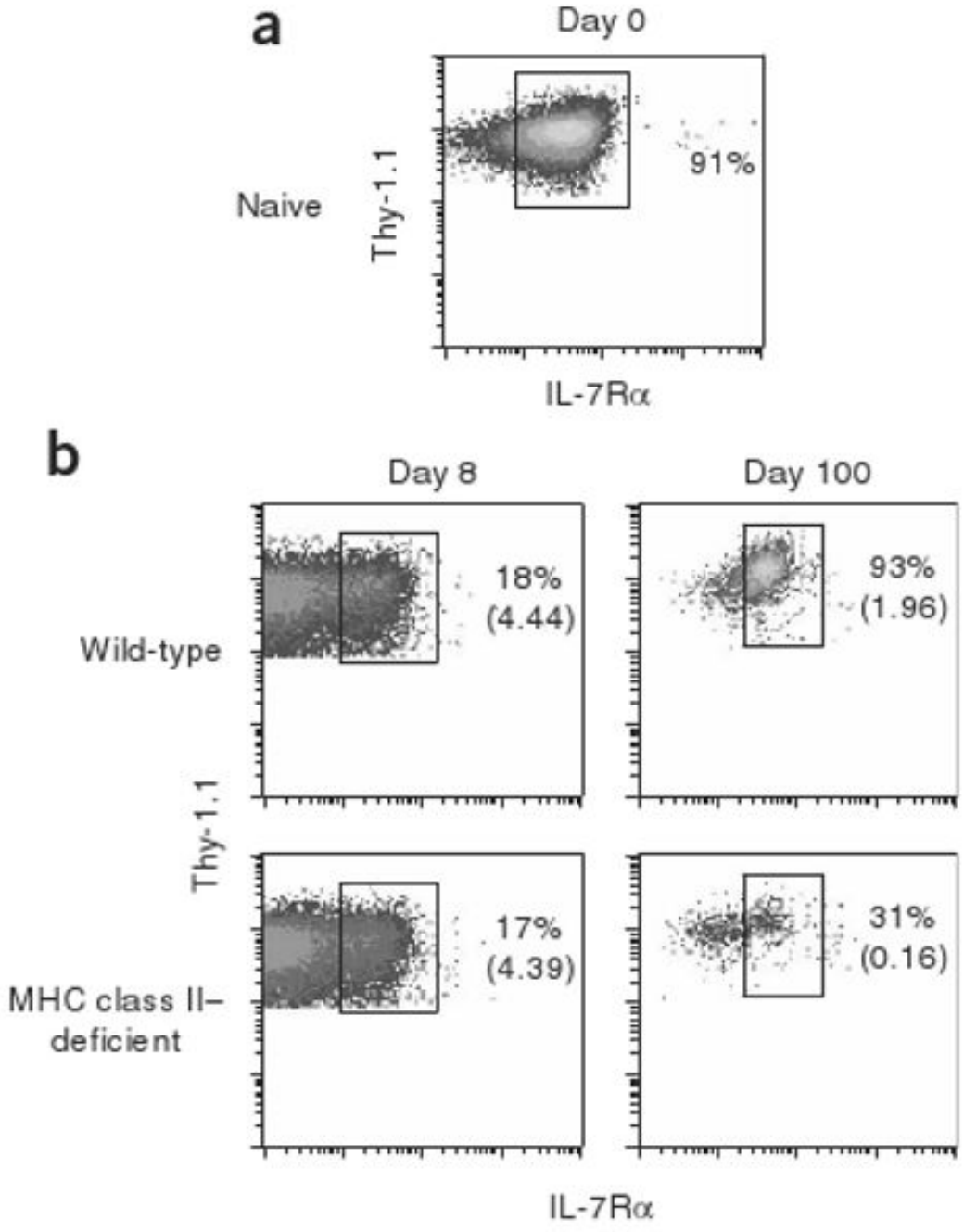

Figure 2.

Changes in IL-7Rα expression on $CD8^+$ T cells during the programming and memory phases in wild-type and MHC class II–deficient mice. (**a**) Surface IL-7Rα expression on naive, $CD8^+$ T cell–enriched P14 cell populations. Number beside boxed area indicates the percentage of IL-7Rα^{hi} cells in the $CD8^+$ T cell population. (**b**) Wild-type and MHC class II–deficient mice containing 5×10^4 P14 cells were immunized with LCMV, and day-8 effector and day-100 memory $CD8^+$ T cells specific for GP(33–41) (using anti-Thy-1.1 staining) were analyzed for surface expression of IL-7Rα. Plots are gated on $CD8^+$ splenocytes; numbers beside boxed areas indicate the percentage of IL-7Rα^{hi} cells within the GP(33–41)-specific $CD8^+$ T cell populations (top number) and in the total spleen population (bottom number in parentheses).

Data are representative of three independent experiments, with three to five mice per group at each time point.

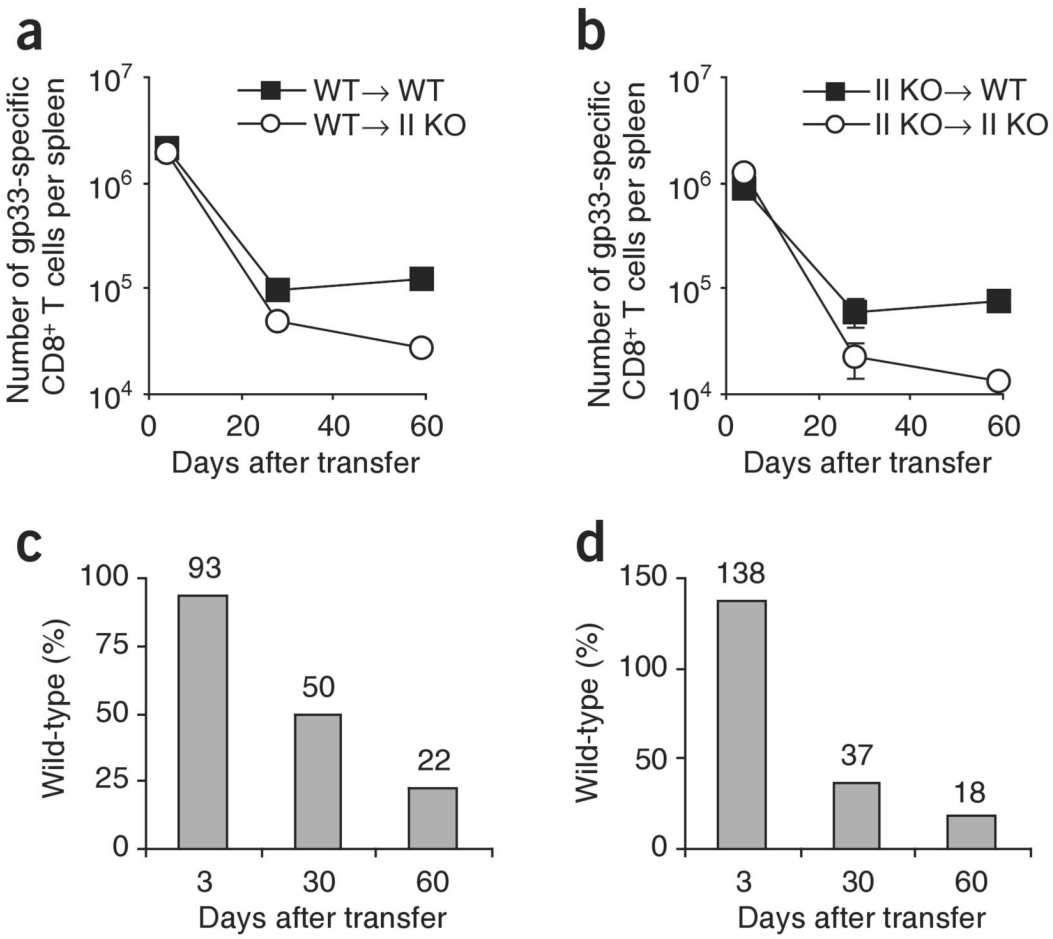

Figure 3.

Transfer of effector $CD8^+$ T cells demonstrates that $CD4^+$ T cells are required after, but not during, the programming phase for the development of stable CD8 memory. (**a,b**) LCMV-specific effector $CD8^+$ T cells at 8 d after infection from wild-type (WT) mice (**a**) or MHC class II–deficient (II KO) mice (**b**) were adoptively transferred into (→) wild-type or MHC class II–deficient secondary recipients. In the primary host, naive $CD8^+$ T cell populations underwent programming and expanded to effector cell populations, which were then isolated and enriched for $CD8^+$ T cells. Approximately 5×10^6 to 7×10^6 effector cells were adoptively transferred to all secondary recipients to undergo contraction and memory maintenance. Secondary recipients receiving transferred cells were analyzed 3, 30 or 60 d later, and absolute numbers of antigen-specific cells in the whole splenocyte population were calculated. Data are presented as the mean ± standard deviation of two to four mice per group at each time point. (**c,d**) Percentage of memory $CD8^+$ T cells in MHC class II–deficient versus wild-type secondary recipients at different days after receiving effector $CD8^+$ T cells from wild-type (**c**) or MHC class II–deficient (**d**) primary hosts. Numbers above bars indicate percentage of $CD8^+$ memory T cells in MHC class II–deficient versus wild-type mice. Results are representative of three independent studies.

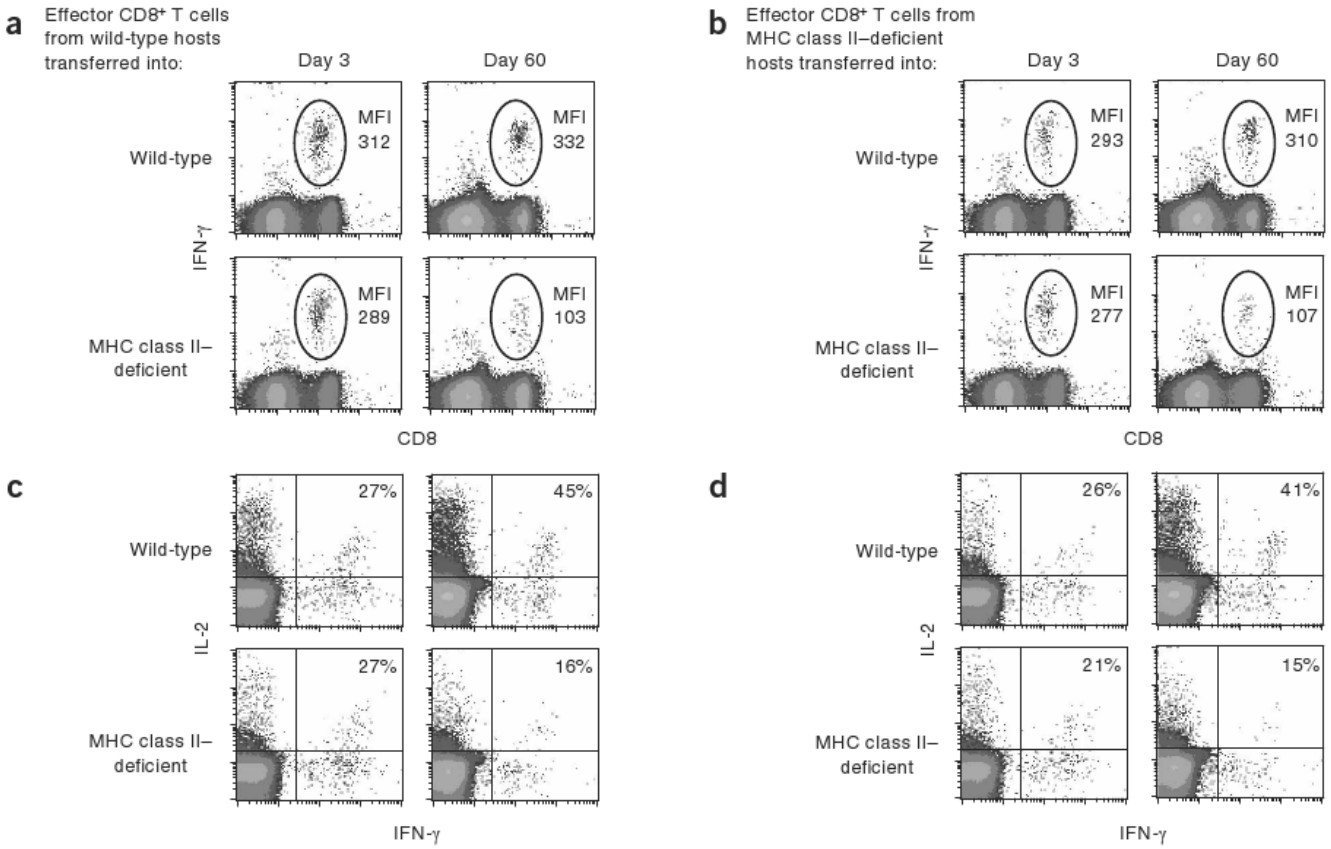

Figure 4.

Effector CD8$^+$ T cells transferred to MHC class II–deficient mice become functionally impaired. LCMV-specific effector CD8$^+$ T cells at 8 d after infection from wild-type (**a,c**) or MHC class II–deficient (**b,d**) primary hosts were adoptively transferred into wild-type or MHC class II–deficient secondary recipients. Transferred cells were analyzed at days 3 and 60 after transfer for production of IFN-γ and IL-2 after 5 h of stimulation with GP(33–41) peptide. (**a,b**) Mean fluorescence intensity (MFI) for IFN-γ staining in gated region. (**c,d**) Percentages of IL-2-producing cells in the total IFN-γ-producing CD8$^+$ T cell population. Data are representative of three independent studies, with two to four mice per group at each time point.

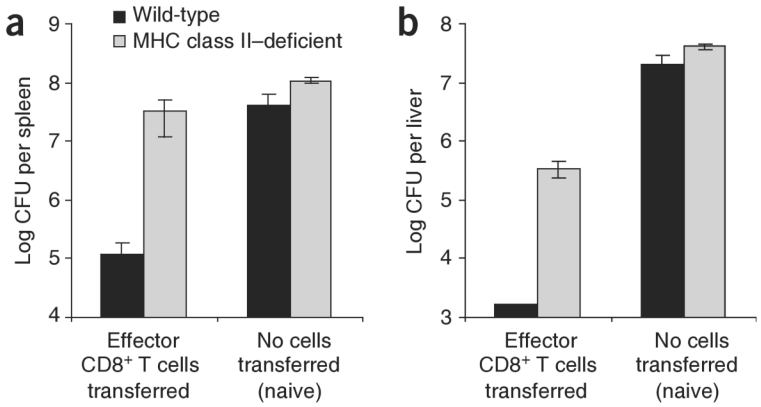

Figure 5.

Effector $CD8^+$ T cells transferred to MHC class II–deficient mice are unable to confer protection against bacterial challenge. Effector $CD8^+$ T cells generated in wild-type primary hosts were transferred into wild-type or MHC class II–deficient secondary recipients and, on day 60 after transfer, mice were challenged with 2×10^5 rLmGP. Naive mice (no effector $CD8^+$ T cells transferred) were challenged as controls. Bacterial clearance was measured by determination of the average colony-forming units (Log_{10} CFU) 72 h after rLmGP challenge in spleen (**a**) and liver (**b**). Data are presented as the arithmetic mean ± standard deviation of two to four mice per group and are representative of two independent studies.

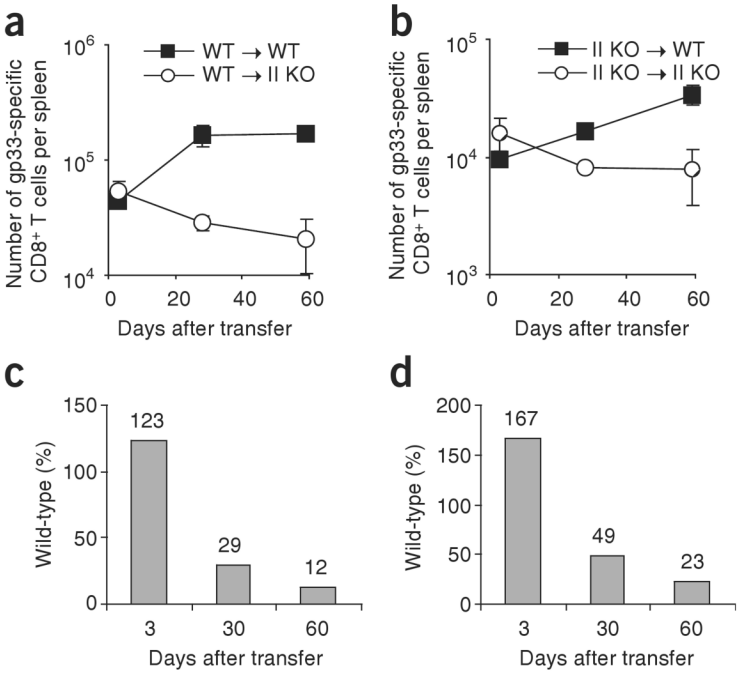

Figure 6.

Transfer of memory cells demonstrates that $CD4^+$ T cells are required for the maintenance of $CD8^+$ T cell memory. LCMV-specific memory $CD8^+$ T cells at day 35 after infection from wild-type mice (**a**) or MHC class II–deficient mice (**b**) were adoptively transferred into wild-type or MHC class II–deficient secondary recipients. In the primary host, naive $CD8^+$ T cell populations underwent programming and contraction and became memory cell populations, which were then isolated and enriched for $CD8^+$ T cells. Memory cells (1×10^6 to 2×10^6) were adoptively transferred into secondary recipients for examination of the maintenance of $CD8^+$ T cell memory. Secondary recipients receiving transferred memory cells were analyzed 3, 30 or 60 d later, and absolute numbers of antigen-specific cells in the whole splenocyte population were calculated. Data are presented as the mean ± standard deviation of two to four mice per group at each time point. (**c,d**) Percentage of memory $CD8^+$ T cells in MHC class II–deficient versus wild-type secondary recipients at different days after receiving memory $CD8^+$ T cells from wild-type (**c**) or MHC class II–deficient (**d**) primary hosts. Numbers above bars indicate percentage of $CD8^+$ memory cells in MHC class II–deficient versus wild-type mice. Results are representative of two independent studies.