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The Development of Functional CD8 T Cell Memory after Listeria monocytogenes Infection Is Not Dependent on CD40

Megan J. Montfort,* H. G. Archie Bouwer,*‡§ Cynthia R. Wagner,‡§ and David J. Hinrichs*‡§

The immunologic requirements for generating long-lived protective CD8 T cell memory remain unclear. Memory CD8 populations generated in the absence of CD4 T cells reportedly have functional defects, and at least a subset of CD8 T cells transiently express CD40 after activation, suggesting that direct CD4+CD8− T cell interactions through CD40 may influence the magnitude and functional quality of memory CD8 populations. To ascertain the role of CD40 in such direct T cell interactions, we investigated CD8 T cell responses in CD40−/− mice after infection with Listeria monocytogenes, an intracellular bacterium that induces APC activation and thus priming of CD8 T cells independently of CD4 T cell help through CD40. In this study we show that memory CD8 T cells generated in CD40-deficient mice show in vivo cytotoxicity and cytokine production equivalent to CD8 memory T cells from wild-type mice. Upon secondary Listeria infection, CD40−/− memory CD8 T cells expand to greater numbers than seen in wild-type mice. These results indicate that CD40 ligation on CD8 T cells, although reportedly a part of CD8 T cell memory development in an H-Y-directed response, is not needed for the development of functional memory CD8 T cell populations after Listeria infection. The Journal of Immunology, 2004, 173: 4084–4090.

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D4 Th cells are critical for CD8 T cell activation and the in vivo development of effector function in many settings. Through CD40-CD40L interactions, CD4 T cells provide help to developing CD8 T cells responses indirectly by triggering APC maturation (1), which renders APC capable of stimulating the differentiation of naive CD8 T cells into effector and memory populations. This method of APC activation is notably important for priming CD8 T cell responses to Ags presented in the absence of infection or inflammation, such as tumor-specific and cross-presented Ags (2–4). In contrast, priming of naive CD8 T cells to many viral or bacterial infections, including the systemic infection.

In addition to influencing CD8 T cell priming, CD4 Th cells strongly influence CD8 memory function, even in infectious settings not reliant on CD40-mediated APC maturation signals. In these models, memory CD8 T cells generated in the absence of CD4 T cells show qualitative defects, including attrition of memory cell numbers, decreased proliferation to Ag, and impaired effector cytokine production on a per cell basis (19–21). It is unclear whether these memory defects are due to the absence of established mechanisms of CD4 Th cell help (CD40 signals and/or cytokine production) or a consequence of other interactions. Recent data have shown that CD4 T cells specific for the male Ag in the absence of CD4 T cells generates memory CD8 T cells that proliferate poorly upon secondary exposure to Ag (22). This work has lead to the hypothesis that CD4 T cells influence the quality of developing CD8 memory populations through CD40 ligation directly on CD8 T cells. To address whether CD40 ligation is a global requirement for generating functional memory CD8 T cells, we investigated the quality of memory CD8 T cell responses after Listeria infection in CD40−/− mice.

We report in this study that the absence of CD40 during CD8 T cell priming has no functional consequence on the quality of either the effector or memory CD8 T cells generated after Listeria infection. In contrast, CD40-deficient mice generate secondary CD8 T cell responses larger in magnitude than those in wild-type mice. These results indicate that direct CD4–CD8 T cell interactions through CD40 and CD40L are not required for the development of functional and long-lived CD8 T cell memory populations after Listeria infection.

Materials and Methods

Mice and cell lines

BALB/cJ and CD40−/− (CNCr.129P2-Tnfrsf5tm1Kik) mice were purchased from The Jackson Laboratory (Bar Harbor, ME). CD40−/− mice were maintained by in-house breeding at the Veterans Affairs Medical Center. The absence of CD40 expression in CD40−/− mice was routinely confirmed by flow cytometry. All experiments with animals were conducted under approval of the Veterans Affairs institutional animal care and use
Mice were systemically infected by i.v. injection with \(1 \times 10^6\) CFU (10 LD_{50}) of \(L.\) monocytogenes in the lateral tail vein in a volume of 1 \(\mu\)l of \(L.\) monocytogenes strain 32531 (American Type Culture Collection, Manassas, VA) was used in all experiments. Unless otherwise noted, memory cells were rested for 4 wk before primary \(L.\) infection and secondary challenge infection with \(1 \times 10^3\) CFU (10 LD_{50}).

**Peptides**

Synthetic peptides for listeriolysin O_{91-99} (LLO_{91-99} = GYKDGNEYI) and p60_{217-225} (GYKVSVQVD) were produced at the Veterans Affairs Medical Center using a Synergy apparatus (Applied Biosystems, Foster City, CA) and standard F-moc chemistry.

**Isolation of lymphocyte populations**

Spleens were removed, and single-cell suspensions were prepared by passing the tissue over a cell strainer. For evaluation of lymphocytes from lung and liver tissues, anesthetized mice were perfused with 10 ml of PBS and 75 \(\mu\)l heparin (Sigma-Aldrich). Lymphocytes were collected from liver tissue by passing the cells over a strainer, then centrifugation through 35% Percoll containing 100 \(\mu\)l heparin (6).

**ELISPOT assay**

Ninety-six-well nitrocellulose plates (Millipore, Bedford, MA) were coated overnight at 4°C with capture \(\alpha\)-IFN-\(\gamma\) Ab (mouse IFN-\(\gamma\) ELISPOT pair; BD Pharmingen, San Diego, CA), then blocked for 2 h with RPMI 1640-10% FCS. RMAS-K4 cells were kept overnight at room temperature, pulsed with 10^{-6} M peptide for 2 h, and then washed thoroughly. Peptide-pulsed RMAS cells and 1–50 \(\times\) 10^5 splenocytes from \(L.\) Listeria-infected mice were added to wells in a total volume of 200 \(\mu\)l and cultured for 20 h at 37°C. All wells were set up in duplicate. Plates were developed according to the manufacturer’s protocol, and spots were visualized using BCIP/NBT substrate (Kierkegaard & Perry Laboratories, Gaithersburg, MD). The number of spots per well was assessed with an Axioplan 2 microscope (Zeiss, Oberkochen, Germany) and KS ELISPOT software (Zeiss).

**Listeria CFU clearance assay**

To evaluate the clearance of primary and challenge \(L.\) infections, the bacterial burdens in the spleens and/or livers was determined by an ex vivo CFU clearance assay as previously described (24). Briefly, spleens and livers from infected mice were harvested and homogenized mechanically, then serial dilutions were plated onto BHI agar and incubated overnight at 37°C. The log_{10} CFU burden per gram of tissue was calculated as: log_{10} [(CFU/dilution factor) \times (organ weight + homogenate volume/organ weight)].

**Intracellular cytokine staining, avidity analysis, and flow cytometry**

Intracellular cytokine staining was performed by culturing cells ex vivo for 5 h with 10^{-5} M peptide in the presence of brefeldin A. Surface staining for CD8 and intracellular staining for IFN-\(\gamma\) and TNF-\(\alpha\) were performed with the Cytofix/Cytoperm kit according to the manufacturer’s directions (BD Pharmingen). Cells were stained with FITC-, PE-, PE-Cy7-, and/or allophycocyanin-conjugated Abs to CD45R2 (clone 53-5.8; BioLegend, San Diego, CA), CD4 (clone RM4-5; BD Pharmingen), CD25 (clone PC61; BD Pharmingen), IFN-\(\gamma\) (clone XMG1.2; BioLegend), and TNF-\(\alpha\) (clone MP6-XT22; eBioscience). Data acquisition was performed on a FACSCompair flow cytometer, and data were analyzed using CellQuest software (BD Biosciences, Mountain View, CA).

The avidity of the responding CD8 T cell population was determined by performing intracellular cytokine staining on spleen cells cultured ex vivo with a gradient of LLO or p60 peptides as described above. For each animal, the frequency of IFN-\(\gamma\)-producing CD8 T cells in response to each peptide concentration was determined, then standardized, setting the largest response in each mouse at 100%. The avidity of each peptide-specific response was determined to be the concentration of peptide required to stimulate 50% of the potentially responsive CD8 T cells from individual animals (25).

**In vivo cytotoxicity assay**

The analysis of in vivo clearance of peptide-loaded target cells was performed as previously described (26, 27). Briefly, naive BALB/c splenocytes were labeled with PKH26 (Sigma-Aldrich), and 1 \(\mu\)M, 100 \(\mu\)M, or 1 nM CFSE (Molecular Probes, Eugene, OR). Labeled cells were pulsed with 1 \(\mu\)M LLO_{91-99} p60_{217-225} or no peptide, for 1 h at room temperature. Each target cell population (5 \(\times\) 10^5) was injected i.v. into recipient mice. Animals were rested 6 h before recipient spleens were analyzed by FACS for target cell clearance. Gating on PKH26+ cells, the percent killing was calculated as: 100% - [(% peptide pulsed in immune% unpulsed in immune) / (% peptide pulsed in naive% unpulsed in naive)] \times 100.

**Data analysis**

Data are expressed as the average \pm SD, and a representative experiment is shown for each figure. Statistical probabilities were evaluated by Student’s t test, with a value of \(p < 0.05\) considered significant.

**Results**

The CD8 T cell response to systemic infection with \(L.\) monocytogenes has been extensively characterized in H-2d mice (BALB/c), with the majority of the cells responding to the H-2Kd-presented immunodominant epitopes LLO_{91-99} (from LLO, a secreted virulence factor), and p60_{217-225} (from the murine hydrolase p60) (28, 29). Previous work has shown that the absence of CD40-CD40L interactions during \(L.\) infection has a minimal impact on the generation of CD8 T cell memory populations, such that the only deficiency is a slight reduction in the size of the memory CD8 population maintained after primary infection (5, 6, 30). The majority of these studies were conducted in CD40-deficient mice or in systems where CD40-CD40L interactions are prevented by in vivo administration of blocking Abs. Memory CD8 T cells are capable of providing protection against challenge infection in the absence of CD4 Th cells (31, 32), making the \(L.\) infection model particularly attractive for assessing the contribution of CD40 signals to the quality of memory CD8 T cell populations.

**CD40 deficiency does not influence the primary CD8 T cell response to Listeria**

To determine whether the absence of CD40 impaired the clearance of \(L.\) Listeria response to primary acute infection, the bacterial load in individual mice was assessed throughout the primary anti-\(L.\) response by CFU clearance assays. As shown in Fig. 1A, the number of bacteria recovered from infected mice was comparable between the wild-type and CD40^{-/-} hosts at all time points. By day 10 after infection, no bacteria could be detected in either mouse strain (data not shown). These results are in agreement with previous work, indicating that CD40-CD40L interactions are not required for the resolution of a primary systemic \(L.\) infection (5, 7, 33).

To evaluate the cytolytic activity of effector cells generated in the absence of CD40 after primary infection with \(L.\) Listeria, we performed in vivo cytotoxicity assays in wild-type and CD40^{-/-} mice (26, 27). On day 7 after primary infection, mice were infused with target cells coated with either LLO_{91-99} or p60_{217-225} peptides, and the clearance of these target cells from the spleen was evaluated 6 h later by flow cytometry. As shown in Fig. 1B, no significant differences were found in the ability of wild-type or CD40^{-/-} mice to clear either target populations.
The development of CD8 T cell responses to infection follows a conserved kinetic pattern of three phases. After Listeria infection, this process appears as an initial expansion of pathogen-specific CD8 T effector cells, which reach a peak number in the spleen on day 7. These cells then undergo a contraction phase, with ~75% of Ag-specific CD8 T cells being eliminated from the spleen by day 10, leading to the establishment of a stably maintained pool of resting memory CD8 T cells by day 30 after infection (34). To evaluate the contribution of CD40 to the generation of functional CD8 effector T cell populations, we examined the kinetics of the primary CD8 T cell responses to the LLO91-99 and p60217-225 epitopes in wild-type and CD40-/- mice after systemic infection with Listeria. Throughout the expansion, peak, and contraction phases of the primary CD8 T cell response, the number of peptide-specific CD8 T cells was determined by ELISPOT analysis (Fig. 2). Although the onset of the contraction phase for both LLO91-99- and p60217-225-specific responses appeared to be slightly delayed in CD40-/- mice, the magnitude of expansion of both peptide-specific populations was equivalent between wild-type and CD40-/- mice. In addition, the number of epitope-specific CD8 T cells maintained as a memory population on day 30 postinfection was also equivalent between wild-type and CD40-/- mice. Taken together, these experiments indicate that CD40-/- mice are capable of generating functional effector CD8 T cells equivalent to those in wild-type mice after primary Listeria infection.

**CD40 deficiency reduces the avidity of memory CD8 T cells**

Blockade of CD40-CD40L signaling during Listeria infection results in a slight reduction in the number of memory CD8 T cells (30); however, a detailed assessment of the functional capabilities of these cells has not been performed. To evaluate the influence of CD40 on the functional properties of CD8 T cell memory populations, we examined the TCR avidity of the LLO91-99- and p60217-225-specific memory CD8 T cells in wild-type and CD40-/- memory mice to cognate peptide. Mice were infected with Listeria, then rested for 4 wk before receiving a secondary challenge infection. On day 5 after the secondary Listeria challenge, splenocytes were cultured ex vivo with a gradient of LLO91-99, or p60217-225 peptide concentrations, then stained for intracellular accumulation of IFN-γ or TNF-α. The avidity of each peptide-specific response was determined to be the concentration of peptide required to stimulate IFN-γ production by 50% of the potentially responsive CD8 T cells from individual animals (25). As shown in Fig. 3, A and B, the avidity of the LLO91-99- and p60217-225-specific populations from CD40-/- mice was reduced in comparison with wild-type mice. However, when we evaluated the quantity of intracellular IFN-γ or TNF-α accumulated on a per cell basis by these same CD8 T cell populations, we saw no differences between memory CD8 T cells generated in CD40-deficient and wild-type mice (Fig. 3, C and D). Together, these results indicate that the absence of CD40 during either the priming phase or the secondary response may influence the sensitivity of the TCR to Ag. However, once stimulated, CD40-/- CD8 memory T cells produce equivalent effector cytokines as their wild-type counterparts.

**FIGURE 1.** CD40-/- mice show normal cytotoxic function after primary Listeria infection. Wild-type (+/+) and CD40-deficient (-/-) mice were infected with Listeria. A, Bacterial burdens were then determined by CFU clearance assays on livers and spleens on days 2, 4, and 7 after infection, and are reported as the log10 number of CFU per gram of tissue. Data are representative of three experiments with two to four mice per group. ND, none detected. B, In vivo killing assays were performed to determine the ability of CD8 primary effector T cells to clear LLO91-99 or p60217-225-coated target cells on day 7 after primary Listeria infection. Data are representative of two experiments with two to four mice per group.

**FIGURE 2.** CD40 deficiency does not impair CD8 T cell priming to Listeria. Wild-type (+/+) and CD40-deficient (-/-) mice were infected with Listeria, and the numbers of LLO91-99-specific (A) and p60217-225-specific (B) CD8 T cells per spleen were evaluated by ELISPOT on days 4, 7, 10, and 30. Data represent two experiments with two to four mice per time point.
CD40 deficiency does not impair the effector function of memory CD8 T cells

One hallmark of functional Listeria-specific memory CD8 T cells is their ability to provide protection against an otherwise lethal challenge infection. To determine the protective capacity of the memory CD8 population generated in the absence of CD40, the clearance of bacteria from wild-type and CD40−/− 4-wk memory mice was analyzed 2 days after Listeria challenge by CFU clearance assay (24). As shown in Fig. 4A, both wild-type and CD40−/− memory mice were capable of providing significant anti-Listeria protection compared with naive mice. Within 4 days of challenge, both wild-type and CD40−/− memory mice had completely cleared Listeria from the spleen, whereas naive mice would succumb to this challenge dose within this time frame. This confirms that memory CD8 T cells generated in the absence of CD40 signals are capable of providing protective immunity, and that the reduced TCR avidity observed in CD40-deficient mice does not impair protection against a secondary Listeria infection.

To evaluate more closely the cytotoxic function of memory CD8 T cells generated in the absence of CD40, we performed in vivo cytotoxicity assays with wild-type and CD40−/− memory mice that had been rested for either 4 or 8 wk after primary Listeria challenge. Memory wild-type and CD40−/− mice rested either 4 wk (B) or 8 wk (C) after primary Listeria infection. Data are representative of two experiments with four mice per group. ND, none detected. **, p < 0.019 vs wild-type mice.

CD40 deficiency does not impair the avidity, but not the cytokine production, of memory CD8 T cells

Wild-type (+/+ ) and CD40-deficient (−/−) 4-wk memory mice were assessed 5 days after secondary Listeria challenge for the avidity of LLO91–99 (A) and p60217–225-specific CD8 T cells (B) by intracellular cytokine staining for IFN-γ. The geometric mean fluorescence intensities (gMFI) of intracellular IFN-γ (C) and TNF-α (D) were determined for LLO91–99- and p60217–225-specific CD8+ T cells. Data are representative of two experiments with four mice per group. *, p < 0.02 vs wild-type mice.
infection. In mice rested for 4 wk after *Listeria* infection, wild-type and CD40−/− CD8 T cells showed equivalent clearance of LLOp60217-pulsed target cells (14.6% wild type vs 13.4% CD40−/−), as well as p60217-bearing targets (9.5 vs 12.8%; Fig. 4B). In 8-wk memory mice, clearance of LLOp60217-pulsed target cells was also equivalent between wild-type (6.0%) and CD40-deficient (5.8%) mice. However, after resting 8 wk, p60217-specific clearance was only apparent in CD40−/− hosts (6.2%; Fig. 4C). Interestingly, the ability to clear peptide-coated target cells within a 6-h assay continued to decrease in both wild-type and CD40−/− mice between wk 4 and 8 postinfection. However, we found no setting in which the absence of CD40 expression negatively affected cytolytic function of memory CD8 T cells. In contrast, the absence of CD40 resulted in longer maintenance of p60217-specific CD8 cytotoxic function.

CD40−/− mice show enhanced CD8 T cell expansion to a secondary *Listeria* infection

To determine whether the absence of CD40 impacts the proliferative potential of memory CD8 cells, we evaluated the in vivo expansion of CD8 memory T cell populations after secondary *Listeria* challenge. Wild-type and CD40−/− mice were rested for 4 wk between primary and challenge infections, then evaluated 5 days postchallenge by intracellular cytokine staining for IFN-γ to evaluate the magnitude of numerical expansion of LLOp60217- and p60217-specific CD8 T cell populations. When the frequencies of peptide-specific CD8 T cells in the spleen were assessed, the LLOp60217-specific populations were not significantly different between wild-type and CD40−/− mice. In contrast the frequency of p60217-specific cells was significantly increased in the CD40−/− mice compared with wild-type (Fig. 5A; *p < 0.0004*). However, when these frequencies were used to calculate the number of peptide-specific cells per spleen in individual mice (Fig. 5B), we found that both the LLOp60217- and p60217-specific populations were significantly increased in the spleens of CD40−/− mice. This observation is not due to a defect in T cell trafficking within the CD40-deficient host, because enhanced numbers of p60217-specific CD8 T cells were also evident in the liver (Fig. 5C) and lung (Fig. 5D) of CD40−/− mice 5 days after secondary *Listeria* challenge. Because the day 30 memory populations specific for these two peptides are equivalent before secondary *Listeria* challenge (Fig. 2), these results indicate that the absence of CD40 does not impair the proliferative potential of memory CD8 T cells generated after a primary *Listeria* infection, but, in fact, contributes to an apparent expansion of peptide-specific CD8 T cells after a secondary challenge infection.

**Discussion**

The interplay among APC, CD4 Th cells, and CD8 T cells leading to the activation and differentiation of naive CD8 T cells into a protective memory population has been extensively studied, and the understanding of how these cells interact will assist the development of future vaccination strategies. Experimental data from several groups have led to the generally accepted APC licensing theory that proposes CD4 Th cells expressing CD40L are able to stimulate immature APC through CD40 ligation to a state capable of priming naive CD8 T cells (2–4). In these models, treatment of APC with anti-CD40 Abs or viral infection also allows the activation of APC to a state that can stimulate CD8 T cells, demonstrating additional pathways of APC licensing.

What happens after T cell priming? The signals that influence primary CD8 effector populations to expand, then contract and subsequently be maintained as stable, protective memory populations are still unknown. Recent reports have suggested that although memory CD8 T cells can be primed in the absence of CD4 Th cells, after conversion to the memory state, these CD8 T cells have functional defects that become apparent upon secondary Ag
exposure (19–22). This may be a consequence of cytokine starva
tion during priming, because CD4 Th cells are known to provide
IL-2 during T cell activation. Alternatively, this could be an effect
of a direct CD4-CD8 T cell interaction that is absent when CD4 Th
cells are not present during CD8 T cell priming. Because CD40
is transiently expressed on a subset of CD8 T cells during activa
tion, direct ligation of CD40 on the surface of CD8 T cells by CD40L-
bearing CD4 Th cells has been proposed as a possible mechanism
of direct CD4-CD8 collaboration and a contributor to CD8 T cell
memory development (22).

Using the Listeria infection model in wild-type and CD40-de
deficient mice, we have evaluated some of the parameters that define
functional memory CD8 T cells: secondary expansion to cognate
Ag, TCR avidity, effector cytokine production, protection against
challenge infection, and in vivo killing of Ag-bearing target cells.
Our data indicate that the majority of these hallmarks of CD8 T
cell memory are expressed fully in CD40-deficient mice after Lis
teria infection, suggesting that direct CD4-CD8 interactions
mediated through CD40 are not required for the generation of func
tional CD8 memory T cells. Although we do observe a small
decrease in CD40+/− memory CD8 TCR avidity, this has no func
tional consequences in our system, as CD40+/− mice are fully
protected against an otherwise lethal secondary Listeria
challenge infection. This is in agreement with a recent report showing
that CD40 expression on CD8 T cells is not required for the generation
of memory CD8 T cells after influenza infection (35).

Recently, Sun and Bevan (36) reported that the generation of
SIINFEKL-specific memory CD8 T cells after infection with re
combinant L. monocytogenes expressing OVA was equivalent in
wild-type, CD40−/−, and CD40L−/− mice on the H-2Kb back
ground. In addition, using mixed bone marrow chimeras contain
ning cells from both wild-type and CD40−/− donors within the
same recipient, they evaluated the CD8 T cell responses to LCMV
and Listeria infection. They found that within the bone marrow
chimeras, both wild-type and CD40−/− CD8 T cells were equally
used in the primary and memory CD8 T cell responses. These
experiments are consistent our conclusions that the absence of
CD40 expression on CD8 T cell populations does not impair the
generation of functional memory CD8 T cells.

Several reports have suggested that priming of CD8 T cells in
mucosal tissues is compromised in the absence of CD40-CD40L
interactions. After oral infection with Listeria, Pope et al. (6)
observed a reduction in tetramer-positive CD8 T cells, particularly
in mucosal tissues; however, an evaluation of the memory CD8 T
cell function was not performed. In addition, priming of mucosal CD8
cells after i.v. vesicular stomatitis virus infection was signifi
cantly reduced, whereas splenic responses were unaffected (37).
Collectively, these reports suggest that the requirement for CD40
expression in priming of mucosal CD8 T cells is 1) not dependent
on the route of infection, and 2) not limited to Listeria
infection. Thus, it is possible that the costimulation requirements for CD8 T
cell priming in the mucosa are inherently different from those in
the spleen and other secondary lymphoid compartments. Impor
tantly, when CD40−/− mice primed with vesicular stomatitis virus
received a secondary challenge infection, the mucosal memory
CD8 T cell population showed an equal or greater fold expansion
compared with wild-type mice (37). This suggests that although
primary mucosal CD8 T cell priming may be dependent on CD40
expression, mucosal memory CD8 T cell populations appear to
proliferate normally in the absence of CD40.

Previous studies have characterized splenic CD8 T cell re
sponses after i.v. Listeria infection in the absence of CD40-CD40L
signals by blocking such interactions through the administration of
anti-CD40L Abs in vivo (30) or by infection of CD40L−/− mice
(5, 33). After in vivo CD40L Ab blockade, the number of resting
CD8 memory T cells recovered was slightly reduced in the ab
sence of CD40-CD40L interactions; however, the functional qual
ity of these cells was not evaluated. In contrast, our data suggest
that the absence of CD40 leads to an increase in the number of
peptide-specific CD8 T cells generated after secondary Listeria
infection. We hypothesize that this observation may be a conse
quence of differences in the CD4+ CD25+ regulatory T cell popu
lations, rather than an effect specific to the immune T cells in
CD40−/− mice. We have confirmed other data in two different
mouse genetic backgrounds that indicate CD40−/− mice have a
marked decrease in the number of CD4+ CD25+ regulatory T cells
(38, 39). As recent data have emerged implicating CD4+ CD25+
regulatory T cells in controlling the in vivo expansion of CD8 T
cells in response to Listeria (40) or HSV infection (41), we are
currently investigating whether CD4+ CD25+ regulatory T cells
generated in a CD40-deficient environment have functional defects
in addition to their numeric reduction.

The numerous mechanisms by which CD40 expression influ
ences adaptive immune responses remain complicated. However,
our results confirm that CD40 expression is not a global require
ment for generating CD8 memory (35) and support a model in
which CD4 Th cells provide help to CD8 responses through APC
 licensing or cytokine production rather than by providing direct
signals to CD8 T cells.

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