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The Impact of Pre-Existing Memory on Differentiation of Newly Recruited Naive CD8 T Cells

Matthew D. Martin,* Thomas C. Wirth, †,‡ Peter Lauer, ‡ John T. Harty, †,‡ and Vladimir P. Badovinac*§,*

One goal of immunization is to generate memory CD8 T cells of sufficient quality and quantity to confer protection against infection. It has been shown that memory CD8 T cell differentiation in vivo is controlled, at least in part, by the amount and duration of infection, Ag, and inflammatory cytokines present early after the initiation of the response. In this study, we used models of anti-vectorial immunity to investigate the impact of pre-existing immunity on the development and differentiation of vector-induced primary CD8 T cell responses. We showed that existing CD8 T cell memory influences the magnitude of naive CD8 T cell responses. However, the differentiation of newly recruited (either TCR-transgenic or endogenous) primary CD8 T cells into populations with the phenotype (CD62L hi, CD27 hi, KLRG-1 low) and function (tissue distribution, Ag-driven proliferation, cytokine production) of long-term memory was facilitated when they were primed in the presence of vector-specific memory CD8 T cells of the same or unrelated specificity. Therefore, these data suggested that the presence of anti-vectorial immunity impacts the rate of differentiation of vector-induced naive CD8 T cells, a notion with important implications for the design of future vaccination strategies. The Journal of Immunology, 2011, 187: 2923–2931.

Naive Ag-specific CD8 T cell precursors respond to pathogen-derived Ags, undergo vigorous clonal expansion, acquire effector functions, and develop into memory populations (1–6). Memory CD8 T cells can protect from reinfection with the same or related intracellular pathogens, and vaccine-evoked CD8 T cells hold great potential for the prevention of infectious diseases (7). New experimental approaches and models to dissect immune responses in health and disease led to increased understanding of the cellular and molecular mechanisms that control the abundance, quality, and maintenance of the memory CD8 T cell pool.

It was shown that postinfection immunization, the increased resistance of immunized (immune) hosts to reinfection correlates with the numbers of memory CD8 T cells in both lymphoid and nonlymphoid tissues (8–10). As a result, much effort has been devoted to identifying approaches that increase the quantity of memory CD8 T cells (2, 3, 7). For instance, for most primary infections, the number of memory CD8 T cells represents 5–15% of the peak effector numbers that are generated during the initial Ag stimulation (11–13), suggesting that the generation of primary memory CD8 T cells might be influenced by the parameters that control effector CD8 T cell expansion and/or survival [e.g., Ag presentation, costimulation, signal three cytokines (2, 14–16)]. In addition, prime-boost protocols (repetitive Ag stimulations) are often used because of their ability to elicit large numbers of memory CD8 T cells (17–19). Although the impact of repeated Ag exposures on memory CD8 T cell differentiation and the level of achieved CD8 T cell-mediated protection are under investigation (18, 20–24), prime-boost regimens represent a viable approach in modulating the quantity of memory CD8 T cells (2, 7, 19).

In contrast, the magnitude of primary Ag-specific CD8 T cell responses might be influenced by the presence of pre-existing immunity to the pathogen or to the vector used for Ag delivery. It was shown that existence of anti-vectorial immunity can limit the expansion of newly evoked naive CD8 T cell responses (25–29), but the impact on differentiation and memory CD8 T cell generation is not known. That is an important question because quality (which can be characterized by phenotype and function of Ag-specific CD8 T cells) together with quantity are defining parameters of memory CD8 T cell responses that can influence the degree of protection. Recent studies suggested that the quality of memory is likely determined during the initial days after Ag recognition (2, 15, 17, 30–32). Interestingly, most experimental conditions that affect effector CD8 T cell expansion also influence the effector to memory CD8 T cell transition. As an example, generation of CD8 T cells with memory phenotype and function can be accelerated if priming and early development of effector CD8 T cells occur under low-inflammatory conditions [e.g., peptide-DC immunizations, immunization with Ag-coated microspheres (17, 33, 34)] or when the duration of infection and/or functional Ag presentation is modulated [e.g., antibiotic treatment to stop bacterial infection (30, 31, 35)].

In this study, we analyzed the consequences of existing CD8 T cell-mediated anti-vectorial immunity on the differentiation of newly recruited naive CD8 T cell responses. We showed the presence of memory CD8 T cells of the same or unrelated Ag specificity (anti-vectorial immunity) impacted differentiation of naive CD8
T cell responses (either TCR transgenic [TCR-Tg] or endogenous). Those cells primed in the presence of memory acquire memory-like phenotype and function at an accelerated rate compared with primary CD8 T cell responses primed in a naive (nonimmune) environment. Our results provide new insights into the development of primary CD8 T cell responses in immune mice and might have important implications for the design of vaccination regimens.

Materials and Methods

Mice, bacteria, and virus infections

C57BL/6 mice were obtained from the National Cancer Institute. OT-I transgenic mice were described previously (36, 37). Pathogen-infected mice were housed in the appropriate biosafety conditions. All mice were used at 6–12 wk of age. All animal protocols followed approved Institutional Animal Care and Use Committee protocols. Attenuated ΔA-deficient *Listeria monocytogenes* strains DP-L1942, Ova257-expressing strains, and Ova257/GP33-expressing strains (Att LM, Att LM-OVA, and Att LM-OVA/GP33, respectively) were grown, injected i.v., and quantified, as described (38, 39). Att LM-OVA expresses a secreted OVA fusion protein; the GP33–41 epitope was added to OVA by oligonucleotide-directed PCR to generate Att LM-OVA/GP33. OVA constructs cloned into pPL2 and integrated at the *rNAvg* locus of the *L. monocytogenes* chromosome, as described (40). As a measure of bacterial clearance, CFU were determined in the spleen and liver on the indicated days postinfection (p.i.), as described (41). Vaccinia virus (VacV) expressing the OVA257 peptide (VacV-OVA), the GP33 peptide (VacV-GP33), or the β-Gal peptide (VacV–β-Gal) (42, 43) were injected i.p into the mice.

Adoptive-transfer experiments and isolation of lymphocytes from tissues

OT-I cells were obtained from peripheral blood samples of 2–3-mo-old naive Thy1.1 OT-1 mice. Contaminating memory phenotype (CD44hi/CD11a+ VacV–βGal) (42, 43) were injected i.p into the mice.

Abs and peptides

The following mAbs from eBioscience, with the indicated specificity and with appropriate combinations in fluorochromes, were used: CD8 (clone 53-6.7), Thy1.1 (OX-7 and HB51), Thy1.2 (53-2.1), CD127 (A7R34), CD62L (MEI-14), CD27 (LG.7F9), KLRG-1 (2F1), IFN-γ (XMG1.2), TNF-α (MP6-XT22), IL-2 (JES6-5H4), and appropriate isotype controls. Synthetic OVA257–264 and GP33–41 peptides were used, as previously described (17).

Quantification of CD8 T cell responses, intracellular cytokine staining, and serum cytokine detection

OT-I T cell responses in peripheral blood and tissues were monitored by FACS analysis for Thy1.1+ CD8 T cells. Thy1.2 expression was used to discriminate between primary and secondary responses in the same host. Endogenous Ova257–specific CD8 T cells were detected by allophycocyanin-conjugated tetramer complexes, as described (22). The percentage of CD8 T cells producing cytokines after stimulation with Ova257–264 or GP33–41 peptides was determined using intracellular cytokine staining for IFN-γ and TNF or IL-2 after 5 h of incubation in brefeldin A with or without the indicated peptides (BD Pharmingen) (45). For detection of serum cytokines, serum was collected 24 h after Att LM-OVA infection, from peripheral blood and incubated with beads coated with cytokine-specific Abs, as well as biotin-conjugated secondary Abs, as recommended in the Mouse Th1/Th2 10plex Kit FlowCytomix manual (eBioscience). Samples were then incubated with streptavidin-PE solution before being resuspended in Assay Buffer and analyzed on a FACSCalibur flow cytometer.

Statistical analysis

Statistical significance was assessed using the two-tailed *t* test, with a confidence interval >95%.

Results

Pre-existing memory CD8 T cells influence differentiation of naive CD8 T cell responses of the same specificity postinfection

To test the idea that the presence of primary memory CD8 T cells can influence differentiation of naive CD8 T cell responses, we adoptively cotransferred physiological numbers of naive TCR-Tg OT-I D8 T cells [500 cells/recipient; Thy1.1/1.1 (36)] with different numbers of primary memory OT-I cells (3 × 10^5 or 5 × 10^5 cells per recipient; Thy1.1/1.2) (Fig. 1A, experimental design). All groups of naive C57BL/6 (B6; Thy1.2/1.1) recipient mice, including a control group that was initially seeded with naive OT-I cells only, were infected with a sublethal dose (0.1 LD_{50}) of attenuated recombinant Att LM-OVA. At various days p.i., the changes in the expression of phenotypic markers (CD127 [IL-7Rα], CD62L, CD27) known to be modulated during primary infection were examined (Fig. 1B). The results were analyzed by two-way ANOVA followed by post hoc Dunnett test (having *P* < 0.05). The results obtained for each analysis are represented as the mean ± SEM and shaded areas indicate the 95% confidence interval.

FIGURE 1. Primary memory OT-I CD8 T cells influence the phenotype of naive OT-I responses p.i. A, Experimental design. Naive Thy1.1/1.1 OT-I cells alone (naive) or mixed with 3 × 10^5 (low) or 5 × 10^5 (high) primary memory Thy1.1/1.2 OT-I cells were transferred into naive B6 Thy1.2/1.2 recipients 1 d before infection with Att LM-OVA (1 × 10^7 CFU/mouse i.v.). Blood samples were pooled, and the expression of the indicated phenotypic markers was determined in the spleen and liver on the indicated days postinfection (p.i.), as described (41). Vaccinia virus (VacV) expressing the OVA257 peptide (VacV-OVA), the GP33 peptide (VacV-GP33), or the β-Gal peptide (VacV–β-Gal) (42, 43) were injected i.p into the mice.

A

<table>
<thead>
<tr>
<th>Group</th>
<th>Naive OT-I</th>
<th>Memory OT-I</th>
<th>Infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>‘naive’</td>
<td>5x10^5</td>
<td>none</td>
<td>Att LM-OVA</td>
</tr>
<tr>
<td>‘low’</td>
<td>5x10^5</td>
<td>3x10^6</td>
<td>Att LM-OVA</td>
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<tr>
<td>‘high’</td>
<td>5x10^5</td>
<td>5x10^5</td>
<td>Att LM-OVA</td>
</tr>
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B

![CD127, CD62L, CD27](http://www.jimmunol.org/)

C

![CD127, CD62L, CD27](http://www.jimmunol.org/)
memory CD8 T cell development and differentiation (1) were analyzed on responding naive OT-I cells (Fig. 1B, 1C). Phenotype analysis of Thy1.1/1.1 primary OT-I cells at days 12 and 41 p.i. revealed higher percentages of CD127-, CD62L-, and CD27hi-expressing OT-I cells in both groups of mice that contained memory OT-I cells at the time of infection. Importantly, the rate of differentiation of naive OT-I cells into memory was also influenced by the number of pre-existing memory CD8 T cells (Fig. 1B, 1C). Therefore, naive OT-I CD8 T cells responding to infection in the presence of cotransferred memory OT-I converted more rapidly to cells with a long-term memory phenotype (CD127hi, CD62Lhi, and CD27hi).

**Naive TCR-Tg CD8 T cells transferred into recipients with endogenous memory CD8 T cells of the same specificity progress to a CD27hi/CD62Lhi phenotype faster**

To extend our initial observation, additional experiments were performed in which the fate of newly primed naive OT-I cells was followed in previously immunized hosts. In this experimental setup, the differentiation of naive OT-I T cell responses was followed in a more physiologically relevant system because immune host contained endogenous polyclonal primary memory CD8 T cells of the same Ag specificity.

Naive B6 mice were infected with VacV expressing the lymphocytic choriomeningitis virus-derived GP33 peptide (VacV-GP33; without-memory group) or the Ova257 peptide (VacV-OVA; with-memory group) (Fig. 2A, experimental design). At a memory time-point (day 49 p.i.), naive OT-I cells were transferred into both groups of mice before infection with Att LM-OVA. Before the adoptive transfer of naive OT-I cells, the existence of endogenous memory CD8 T cells specific for GP33 and Ova257 after VacV-GP33 or VacV-OVA immunization was confirmed by a peptide-stimulated IFN-γ-secretion assay in the spleen before secondary challenge (Fig. 2B). Importantly, VacV-OVA-immunized mice that contained $\sim 3 \times 10^7$ Ova257-specific CD8 T cells/spleen were able to control and clear secondary L. monocytogenes infection faster than was the VacV-GP33-immunized control group (Fig. 2C). Therefore, this experimental set-up enabled us to investigate how functional endogenous memory CD8 T cells influence the development of newly recruited naive CD8 T cell responses directed against the same epitope.

Primary OT-I CD8 T cell responses were readily detectable in both groups of mice (Fig. 2D) after Att LM-OVA infection. As previously suggested (46), the magnitude of expansion of newly recruited CD8 T cells was decreased in the presence of memory CD8 T cells of the same specificity. Importantly, despite the lower magnitude of the initial expansion, the phenotype of OT-I CD8 T cells at day 7 p.i. was indistinguishable between groups, suggesting that naive to effector CD8 T cell differentiation during the development of primary CD8 T cell responses is not influenced by the CD8 T cell-mediated immunity present at the time of Ag stimulation (Fig. 2E). Consistent with the data presented in Fig. 1, longitudinal analysis of primary OT-I T cell responses in these mice indicated that naive OT-I cells primed in the presence of endogenous polyclonal CD8 T cell memory of the same specificity displayed an accelerated memory differentiation based on increased expression of CD27 and CD62L (Fig. 2F). In addition, it was shown that expression of KLRG-1 is decreased during
differentiation of effector CD8 T cells into memory (15). Importantly, the frequency of KLRG-1<sup>hi</sup> cells was decreased on primary memory OT-I cells initially primed in the immune mice (Fig. 2F).

In summary, pre-existing endogenous CD8 T cell memory influenced the magnitude of expansion of naive CD8 T cells of the same Ag specificity but not their transition from naive to effector populations. Importantly, development of OT-I cells with memory phenotype was facilitated in the immune host, suggesting that endogenous memory CD8 T cell responses might control the developmental program of newly primed CD8 T cell responses.

**Naive endogenous CD8 T cells primed in the presence of vector-specific memory CD8 T cells show decreased levels of expansion but progress to a long-term memory phenotype faster**

In the adoptive-transfer models in which naive TCR-Tg OT-I cells were transferred with primary memory OT-I cells or when they were transferred into recipients with endogenous polyclonal CD8 T cell memory of the same specificity, the differentiation of newly primed OT-I cells into cells with memory phenotype was facilitated (Figs. 1, 2). The next set of experiments was designed to address the extent to which the presence of memory CD8 T cells specific for the vector (anti-vectorial immunity) influences differentiation of primary polyclonal (endogenous) CD8 T cell responses to a new Ag delivered with vector.

Groups of naive B6 mice were immunized with VacV–β-Gal (without-memory group) or VacV-GP33 (with-memory group) 2 mo before secondary challenge with recombinant Att LM-OVA/ GP33 (Fig. 3A, experimental design). GP33-specific memory CD8 T cells were detected in VacV-GP33–immunized mice, and VacV-GP33–immune mice showed increased resistance to secondary LM-OVA/GP33 challenge, indicating that anti-*L. monocytogenes* (anti-vector) immunity was present (Fig. 3B, 3C).

Analysis of the newly primed endogenous Ova257-specific CD8 T cells in control, as well as in immune, mice revealed that the presence of CD8 T cell-mediated anti-vectorial immunity (GP33-specific CD8 T cells) decreased the efficiency of primary Ova257-specific CD8 T cell expansion and subsequent numbers of memory cells (Figs. 3D, 3E). Importantly, despite decreased numbers of endogenous Ova257-specific CD8 T cells generated in the presence of anti-vectorial immunity, those cells converted to a long-term memory phenotype more quickly compared with the cells primed in a nonimmune environment. Longitudinal kinetic analysis of endogenous KbOva257-specific CD8 T cells showed that expression of markers known to be upregulated on Ag-specific memory CD8 T cells (CD127, CD27, CD62L; Fig. 3F) was increased when those cells were primed in the immune hosts. Therefore, naive endogenous CD8 T cells primed in the presence of responding endogenous memory CD8 T cells of unrelated specificity showed decreased levels of expansion but differentiated into a long-term memory phenotype at an accelerated pace.

*The functional consequences of naive CD8 T cell priming in the presence of anti-vectorial immunity*

To address the functional consequences of naive CD8 T cell priming in the presence of anti-vectorial immunity, age- and sex-

**FIGURE 3.** Naive endogenous CD8 T cells primed in the presence of endogenous memory CD8 T cells of unrelated specificity show decreased levels of expansion but progress to a long-term memory phenotype faster. A. Experimental design. Naive B6 mice were infected with either VacV–β-Gal (3 × 10<sup>6</sup> PFU/mouse i.p.; without-memory group) or VacV-GP33 (3 × 10<sup>6</sup> PFU/mouse i.p.; with-memory group); 68 d later, mice were infected with Att LM-OVA/GP33 (1 × 10<sup>4</sup> CFU/mouse i.v.). B. Spleenocytes from mice infected 67 d previously with VacV–β-Gal or VacV-GP33 were incubated in the presence or absence of GP33 peptide and monitored for IFN-γ production. Numbers represent the percentage of Ag-specific IFN-γ<sup>+</sup> CD8 T cells. C. Bacterial titers in the spleen (CFU/spleen) and liver (CFU/g liver) were determined 3 d after Att LM-OVA/GP33 infection. Data are presented as mean ± SD of three mice per group. D. Representative plots showing the frequency of Kb Ova257-specific CD8 T cells at days 7 and 33 p.i. Numbers indicate the percentage of OVA-specific CD8 T cells. E. Percentage of KbOVA257<sup>+</sup> CD8 T cells in PBLs at day 33 p.i. presented as mean ± SD (α = 4 mice per group). F. Kinetic analysis of the expression of the indicated markers for Kb OVA257<sup>+</sup> CD8 T cells. Data are presented as the percentage of cells positive for the indicated marker. The experiment was repeated twice with similar results.
matched naive Thy1.2 B6 mice were either not infected (without-memory group) or infected with a strain of *L. monocytogenes* that does not express the Ova257 epitope (with-memory group). Five hundred naive Thy1.1 OT-I CD8 T cells were transferred into both groups of mice 44 d after initial infection and 1 d before infection with an *L. monocytogenes* strain that expresses the Ova257 epitope (Fig. 4A, experimental design). This experimental set-up enabled us to address the extent to which phenotype (rapid expansion of memory CD8 T cell markers) correlates with changes in function (e.g., tissue distribution, cytokine production, Ag-driven proliferation) for vector-induced primary CD8 T cells primed in the presence of anti-vectorial immunity.

The expansion of newly primed OT-I cells in the blood (Fig. 4B, 4C), secondary lymphoid organs, and tertiary tissues (data not shown) was decreased in mice immune to the vector. Similarly, detailed kinetic analysis in the blood showed that subsequent generation of primary memory CD8 T cell numbers was also decreased in mice that contained pre-existing memory (Fig. 5A). Decreased expansion again correlated with an increase in frequency of CD27hi/CD62Lhi OT-I CD8 T cells 1 mo after Att LM-OVA challenge (Fig. 4E).

To determine whether duration of infection and decrease in inflammation correlate with observed changes in quality and quantities of primary CD8 T cell responses, the kinetics of bacterial clearance and the concentrations of inflammatory cytokines were determined early after secondary infection in both groups of mice (Fig. 5A). The results clearly showed that the bacterial load and duration of infection (Fig. 5B), as well as the amount of inflammatory cytokines (IFN-γ and IL-6) detected in the serum 1 d after secondary challenge (Fig. 5C), were decreased in mice immune to the vector. Thus, these data suggested that priming of naive CD8 T cells in the presence of existing memory will occur in the environment with decreased Ag load and decreased levels of systemic inflammation.

To determine whether primary memory OT-I cells primed in the presence or absence of anti-vectorial immunity showed different patterns of tissue distribution, the representation of OT-I cells in secondary lymphoid organs and tertiary tissues was analyzed 3–4 wk after Att LM-OVA challenge. The frequency of OT-I in total CD8 T cells in each tissue was normalized to the frequency of OT-I in PBLs (Fig. 6A, 6B). The results clearly showed that, consistent with their phenotype, memory OT-I cells primed in immune hosts were significantly enriched (*p* = 0.04) in LNs, but their representation was decreased in lungs (*p* = 0.027) (Fig. 6B). The total number of OT-I cells recovered from LNs was similar in both groups of mice, suggesting that, despite their statistically significant decrease in numbers in the circulation and/or spleen (Fig. 6C), priming of naive CD8 T cells in the presence of memory might not lead to a uniform decrease in memory CD8 T cell numbers in all organs. Therefore, the accelerated differentiation of naive CD8 T cells into memory is coupled with their ability to regain access to LNs.

In addition to the changes in tissue distribution, direct ex vivo Ova257-peptide stimulation of splenocytes 1 mo p.i. revealed a significantly (*p* = 0.02) higher frequency of IL-2–producing IFN-γ*γ* OT-I cells in mice that were immune to the vector (Fig. 7). Similar frequencies of IFN-γ*γ* OT-I cells from both groups of mice also produced TNF after Ova257 peptide ex vivo stimulation (data not shown). Thus, these data suggested that accelerated transition to long-term memory is accompanied by increased frequency of Ag-specific CD8 T cells that are functionally superior in their ability to produce polyfunctional cytokine responses upon Ag stimulation.

**Primary CD8 T cells primed in the presence of anti-vectorial immunity have a greater proliferative potential upon Ag re-encounter.**

One of the defining characteristics of functional memory CD8 T cells is their ability to rapidly expand upon Ag re-encounter and

![FIGURE 4](http://www.jimmunol.org/)
generate increased numbers of secondary effectors that are able to provide protection. To test, on a per-cell basis, whether primary memory OT-I cells primed in naive or immune environments (Fig. 4A, experimental design) differ in their ability to undergo Ag-stimulated proliferation, equal numbers (3 × 10³ per recipient) of positively selected OT-I CD8 T cells were transferred into naive B6 Thy1.2 hosts. A day after adoptive transfer, all groups of recipient mice were infected with Att LM-OVA (1 × 10⁷ CFU/mouse i.v.). Bacterial titers in the spleen (CFU/spleen) and liver (CFU/g liver) were determined 2 and 3 d after Att LM-OVA infection. Data are presented as mean + SD of three or four mice per group. Numbers above bars indicate the fraction of mice analyzed that showed detectable levels of infection (above limit of detection [LOD]). The concentrations of IFN-γ and IL-6 were determined in serum of naive and Att LM-OVA–infected groups of mice 24 h p.i. The results are presented as mean + SD of three mice per group.

**FIGURE 5.** Anti-vectorial immunity decreases the duration of infection and inflammation. A, Experimental design. Naive OT-I cells (Thy1.1, 5 × 10⁶ cells/mouse) were transferred into age-matched naive or previously infected (Att LM) groups of mice 1 d before infection with Att LM-OVA (1 × 10⁷ CFU/mouse i.v.). B, Bacterial titers in the spleen (CFU/spleen) and liver (CFU/g liver) were determined 2 and 3 d after Att LM-OVA infection. Data are presented as mean + SD of three or four mice per group. Numbers above bars indicate the fraction of mice analyzed that showed detectable levels of infection (above limit of detection [LOD]). C, The concentrations of IFN-γ and IL-6 were determined in serum of naive and Att LM-OVA–infected mice of groups 24 h p.i. The results are presented as mean + SD of three mice per group.

**Discussion**

The data presented provided new insight into the process of primary memory CD8 T cell development and differentiation in immune (nonnaive) hosts. What is the physiological rationale of these studies? In other words, how often will naive CD8 T cells have a chance to respond to new Ag in an environment that contains pathogen or vector-specific CD8 T cell memory? First, naive CD8 T cells might be recruited after secondary Ag stimulation when the initial stimulation of naive CD8 T cells was incomplete. Second, naive CD8 T cell precursors will also be produced in the thymus after the resolution of the initial infection. Third, it was also shown that, in the case of chronic viral infections, newly generated CD8 T cells can preserve the viral-specific CD8 T cell pool and become more responsive to boosting strategies (47). Therefore, continuous recruitment of naive CD8 T cells and/or priming of naive CD8 T cell precursors at different times after initial Ag stimulation [e.g., naive CD8 T cells that are recruited later into the response; so-called “latecomers” (48, 49)] might contribute to the heterogeneity and survival (longevity) of the memory CD8 T cell pool. Finally, one has to appreciate the elegance of the immune system, because secondary memory CD8 T cells are more permissive than primary memory for LN priming of naive CD8 T cells of the same Ag specificity (20), suggesting a potential mechanism(s) for memory CD8 T cell replacement, as well as an increase in the complexity of memory CD8 T cell populations, based on Ag-stimulation history (18, 20–24). Therefore, the heterogeneity of the memory CD8 T cell pool might be achieved and maintained when the priming of naive CD8 T cells in the presence of effector and memory CD8 T cells of the same Ag specificity is observed.

In most of the experimental models discussed above, naive and existing memory CD8 T cells were of the same Ag specificity. Probably a more important implication for the studies presented in this article is that accelerated naive to primary memory CD8 T cell differentiation was observed in mice in which naive and existing memory CD8 T cells were of different Ag specificities but were stimulated by the same vector. The importance lies in the fact that a similar scenario can be expected when priming of CD8 T cells against the Ag of choice is carried out in hosts with pre-existing immunity to the Ag-delivery vector.

The idea of using a common vector as a vaccine carrier for multiple immunizations is not new, and potential complications to the approach are well documented. For instance, the efficacy of vaccination with recombinant viral vectors can be greatly diminished by the existence of vector-induced humoral immunity (26, 46, 50). In contrast, *L. monocytogenes* might represent an attractive vaccine-delivery vehicle (46, 51), because the contribution of specific Ab in anti-*L. monocytogenes* protection is minimal (52). However, the impact of pre-existing CD8 T-cell-mediated immunity on the efficacy of *L. monocytogenes* as a vaccine vector has been investigated. In most studies, the existence of anti-*L. monocytogenes* immunity decreased the magnitude of expansion of newly recruited naive CD8 T cells after secondary *L. monocytogenes* challenge (28, 29, 53–55). Although the extent of attenuation of the primary CD8 T cell
responses is likely to be dependent on the quantity and quality of existing anti-"L. monocytogenes" CD8 T cell memory, our data suggested that substantial expansion of naive CD8 T cells can be achieved.

Importantly, because of the difference in duration of infection (bacterial load) and changes in systemic inflammation (14), newly evoked primary CD8 T cells progress to a memory-like phenotype and function at an accelerated rate. Those cells, on the population level, were able to traffic back to LNs, produce effector cytokines, such as IL-2, and they were able to expand in numbers after secondary booster challenge better than were CD8 T cells primed in the naive (nonimmune) environment. Further studies are required to determine the extent to which duration of functional Ag presentation or inflammation controls the development of primary CD8 T cells. Even more importantly, it will be interesting to see whether these findings can be harnessed to increase the efficacy of vector-induced responses when anti-vectorial immunity is present.

Although a correlation between the number of Ag-specific memory CD8 T cells and the level of protection after reinfection is firmly established (8–10), the desired quality of memory CD8 T cells might differ depending on multiple factors, including the nature of the pathogen and route of infection (24, 56, 57). Understanding the factors that influence the balance between the number and quality of vaccine-induced primary CD8 T cell responses would likely speed up identification of vaccination strategies and increase the efficacy of prime-boost regimens that target acute and chronic infections.


