Qualitatively Different Memory CD8+ T Cells Are Generated after Lymphocytic Choriomeningitis Virus and Influenza Virus Infections

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Qualitatively Different Memory CD8+ T Cells Are Generated after Lymphocytic Choriomeningitis Virus and Influenza Virus Infections

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Viral infections often induce robust T cell responses that are long-lived and protective. However, it is unclear to what degree systemic versus mucosal infection influences the generation of effector and memory T cells. In this study, we characterized memory CD8+ T cells generated after respiratory influenza virus infection and compared the phenotypic and functional qualities of these cells with memory T cells generated after systemic infection with lymphocytic choriomeningitis virus (LCMV). Using a recombinant influenza virus expressing the LCMV gp33–41 epitope and TCR transgenic CD8+ T cells with a fixed TCR, we compared responses to the same Ag delivered by mucosal or systemic viral infection. Memory cells generated postinfection with either virus showed only a few phenotypic differences. Yet, influenza memory T cells produced lower amounts of effector cytokines upon restimulation and displayed reduced proliferation compared with LCMV-induced memory cells. Strikingly, we observed reduced expansion of to the same Ag delivered by mucosal or systemic viral infection. Memory cells generated postinfection with either virus showed only a few phenotypic differences. Yet, influenza memory T cells produced lower amounts of effector cytokines upon restimulation and displayed reduced proliferation compared with LCMV-induced memory cells. Strikingly, we observed reduced expansion of

Following activation, T cells undergo a significant phase of proliferation and become effector CD8+ T cells that control infection via production of cytokines and cytolytic molecules, such as perforin and granzymes (1, 2). Contraction of 90–95% of the effector pool upon Ag clearance results in the survival of IL-7Rα–expressing T cells that continue to differentiate into a relatively stable memory population with a heightened capacity for recall (3, 4). Memory T cells are known to be heterogeneous in terms of phenotype and function. Importantly, the events that occur during the priming phase of an immune response, including inflammation, the amount of Ag, duration and strength of TCR signaling, costimulation, CD4 T cell help, and the availability of cytokines, seem to be integral to the quality of the resulting memory cells (5–7).

Memory T cells can persist in tissues after pathogen clearance and mediate more rapid responses against reinfection. In addition to local infections in tissues, such as the mucosa, systemic infections induce memory cells that can home to mucosal tissues, including the gut and the lung (8, 9). Recently, the quality of memory T cells present in different tissues postinfection has begun to be examined in more detail, suggesting a link between T cell quality and protection from disease (10, 11). Yet it remains unclear exactly how the site of viral infection or replication can influence T cell responses. In particular, the type or quality of T cells generated after systemic infection may differ from those primed by a mucosal challenge, such as respiratory viral infection.

In mice infected with the Armstrong strain of lymphocytic choriomeningitis virus (LCMV), systemic infection is controlled by the adaptive immune response, resulting in the formation of highly functional, long-lived populations of memory T and B cells. Similarly, it was demonstrated that respiratory infection of mice with influenza viruses primes considerable immune responses, which are long-lasting (12). However, the generation of long-lived protective immunity against influenza virus infections in humans remains a challenging goal, and influenza viruses cause significant morbidity and mortality annually (13). Ab-mediated mechanisms are effective in providing protection against homologous influenza virus infections; however, they are inadequate against heterologous infections in which the viral proteins, in particular hemagglutinin and neuraminidase (NA), are distinct. This is particularly important when rapid antigenic drift occurs in circulating influenza strains or when attempting to provide protection against potential pandemic strains. Cross-reactive T cell responses are elicited by heterologous infections in humans and may provide protection (12, 14). However, these responses seem to be relatively weak and may not prevent disease in immune individuals, despite the relatively high conservation of T cell epitopes (15, 16), suggesting that such T cell immunity may be suboptimal. Thus, it remains important to characterize the role and effectiveness of heterosubtypic T cell immunity for the design of better influenza virus vaccines.

In this study, we examined the quality of CD8+ T cell memory cells generated after respiratory influenza virus infection and
compared them with memory cells generated after systemic LCMV infection. We generated a recombinant influenza virus expressing the LCMV gp33–41 epitope, allowing us to follow responses to the same Ag after mucosal or systemic viral infection. Using transgenic P14 gp33-specific CD8+ T cells with fixed TCRs, we were able to directly compare effector and memory cells and qualitatively compare recall responses and protective capacity. Memory CD8+ T cells generated by LCMV or influenza virus infection displayed only a few phenotypic differences. However, upon recall, influenza virus–primed memory cells produced lower levels of effector cytokines and displayed reduced proliferative responses and protection from a respiratory challenge compared with LCMV memory cells. This was particularly apparent in memory cells isolated from the lung after influenza virus infection. Together, these data suggest that memory CD8+ T cells generated after influenza virus infection are qualitatively different from those primed by systemic LCMV infection. These data have implications for understanding memory T cell generation and the design of vaccines to elicit optimal T cell responses.

Materials and Methods

Generation of recombinant influenza virus

Recombinant influenza virus was produced using an established eight-plasmid influenza reverse-genetics system (17). The plasmids used in the construction of the recombinant influenza viruses were described previously (18). The LCMV gp33–41 epitope (KAVYNFATM) was inserted into the NA of A/PR/8/34 (H1N1) at residue 42 using a PCR mutagenesis approach. A corresponding number of amino acids (nine - QNHTGICNQ) was deleted from the recombinant viruses to maintain the protein length equal to the wild-type NA protein.

Mice, virus, and infections

Thy1.1+ (B6.PL-Thy1a/CyJ) mice were bred to P14 transgenic mice (19) and maintained in the Emory University animal facility. Splenocytes from naive Thy1.1+ P14 transgenic mice containing 10^6 or 10^5 Ag-specific CD8+ T cells were transferred into 6-wk-old female C57BL/6 (B6) mice (The Jackson Laboratory, Bar Harbor, ME). The following day, mice were infected with 2 × 10^5 PFU LCMV Armstrong i.p., or 200 PFU recombinant influenza virus A/PR/8/34 (H1N1) expressing the LCMV gp33–41 epitope in NA (PR8-33) intranasally. For challenge experiments, mice were intranasally infected with 5 × 10^6 PFU recombinant vaccinia expressing gp33–41 (VV-33) (20). Titters of viral stocks were determined by plaque assay on Vero cells for LCMV (21) or VV-33 (20), as previously described. Influenza virus titers were determined using monolayers of MDCK cells, which were infected with dilutions of virus in DMEM for 1 h at 37°C before overlaying with 1% agarose in DMEM supplemented with 5% FBS and 1 μg/ml N-2-Hydroxyphenylalanine chloromethyl ketone trypsin. Cells were incubated for 3 d and stained with crystal violet (0.1% w/v in 20% ethanol) to count plaques. For determination of viral titers in tissues, lungs or spleens were weighed, and dilutions of homogenized tissue were assayed by plaque assay.

Lymphocyte isolation

Lymphocytes were isolated from tissues as previously described (22). Briefly, bronchoalveolar lavage (BAL) of the airways was performed with three 1-ml washes with PBS before perfusion of the lungs with PBS. Lungs were treated with 1.3 mM EDTA in HBSS (30 min at 37°C, shaking at 200 rpm) and then treated with 100 μM collagenase (Invitrogen Life Technologies, Carlsbad, CA) in 5% RPMI 1640 supplemented with 2 mM CaCl_2 and 2 mM MgCl_2 (60 min at 37°C, shaking at 200 rpm). Single-cell suspensions were obtained by pushing spleens, lymph nodes (LNs), or digested lungs through 70-μM nylon mesh filters (BD Biosciences, San Jose, CA). Lymphocytes from lungs were purified by centrifuging on a 44/67% Percoll gradient (800 × g for 20 min at 20°C).

Abs and flow cytometry

Single-cell suspensions were stained with anti-CD8α–allophycocyanin (53-6.7), Thy1.1-PerCP (OX-7), CD26-L2-FITC (MEL-14), CD27-PE (LG.3A10), CD43-FITC (B11), KLRC1-PE, CD122-PE (TM-b1), CD132-PE (4G3), BCL-2–FITC (3F11), IFN-γ–FITC (XMG1.2), TNF-α–allophycocyanin (MP6-XT22), IL-2–allophycocyanin (JES6-5H4) (all from BD Biosciences), CD127-PE (A7R34) (eBioscience, San Diego, CA), biotinylated IL-15Rα (BAF551) (R&D Systems, Minneapolis, MN), or anti-human granzyme B-PE (Caltag Laboratories, Burlingame, CA). Intracellular staining for granzyme B or BCL-2 directly ex vivo or for IFN-γ, TNF-α, or IL-2 after a 5-h in vitro stimulation with 0.1 μg/ml gp33 peptide (or dilutions of peptide as indicated) was performed using the Cytofix/Cytoperm kit, according to the manufacturer’s instructions (BD Biosciences). Samples were analyzed using a BD Biosciences FACSCalibur.

Memory cell transfers

Thy1.1+ P14 transgenic CD8+ T cells were isolated from the spleens or lungs of mice infected with LCMV or PR8-33 90 d earlier. Naive B6 Thy1.2+ recipient mice (Thy1.2+) received 1 × 10^6 Thy1.1+ CD8+ memory cells to track expansion in the blood or 5 × 10^6 cells to measure protection upon recall with VV-33.

In vitro proliferation assays

Cells were labeled with 1 μM CFSE (Invitrogen Life Technologies) and 7 × 10^5 Thy1.1+ CD8+ P14 cells cultured in a total of 8 × 10^5 splenocytes, alone or in the presence of dilutions of gp33 peptide (10^−7–10^−12 M) or 5 ng/ml recombinant mouse IL-2, IL-7, or IL-15 (R&D Systems) in RPMI 1640 plus 10% FBS for 60 h.

Statistical analysis

Data are expressed as the mean ± SD. Statistical analysis was performed by the two-tailed Student t test with 95% confidence intervals or a one-way ANOVA with a Tukey posttest using Prism software (GraphPad, La Jolla, CA).

Results

Expansion of CD8+ T cells following influenza virus and LCMV infection

Protective cell-mediated immunity to virulent influenza infection requires CD8+ T cells (23). We wanted to examine CD8+ T cell responses to respiratory influenza virus infection and directly compare these with T cells responses after acute systemic LCMV infection, which induces a substantial memory pool (7). To do this, we generated a recombinant influenza virus (A/PR/8/34) expressing the LCMV gp33 epitope in the NA stalk (PR8-33) using an established eight-plasmid influenza reverse-genetics system (17). C57BL/6 mice were adoptively transferred with TCR transgenic P14 CD8+ T cells specific for the LCMV glycoprotein Ag gp33–41. These chimeric mice were then infected with PR8-33 intranasally or LCMV i.p., and expansion of the specific cells was followed over the course of the acute response until the memory phase (day 90). Use of the transgenic P14 CD8+ T cells allowed us to directly compare responses to the different pathogens, because the responding T cell population has a fixed TCR. Thus, differences observed were due to inherent cell properties rather than differences in TCR affinity.

Postinfection, LCMV was found in the spleen, lungs, and LN, and titers peaked around 3 d postinfection and were below the limit of detection within 7–10 d (Fig. 1A, data not shown). Higher titers of influenza virus were observed in the lungs after PR8-33 infection and remained high for ≥6 d. However, no virus was detected in the spleens of these mice at any time, which indicates that the infection remained localized to the respiratory tissues.

Expansion of the gp33-specific CD8+ T cells in the spleen and lungs was considerable after PR8-33 infection. Nevertheless, Ag-specific T cell numbers were most apparent in the lungs, despite considerable more influenza virus (~3 logs), which persisted in the lungs for ≥3 d longer than LCMV (Fig. 1A). These differences in Ag-
specific T cell numbers were maintained for ≥3 mo postinfection. Significant numbers of responding cells were also detected in the draining mediastinal LN soon after PR8-33 infection, although the numbers of memory cells in the LN remained lower in the influenza virus–immune mice than in LCMV–immune mice.

**Phenotypic changes during naive to effector to memory differentiation following influenza virus and LCMV infection**

Responding gp33-specific CD8+ T cells from the spleen, lungs, and LNs of mice infected with LCMV or PR8-33 were analyzed for a number of phenotypic and functional markers (Fig. 2). Down-regulation of CD62L, CD127 (IL-7Rα), and Bcl-2 on the responding P14 T cells during the effector phase, as well as subsequent upregulation during memory differentiation, was very similar after both infections. The costimulatory molecule CD27 was modulated differently in the spleen and lung after PR8-33 infection compared with LCMV, potentially reflecting the duration and level of Ag presentation in these organs. KLRG-1 was upregulated to a higher level after LCMV infection and remained higher on the specific cells for ≥3 mo. KLRG-1 is expressed on short-lived effector cells after LCMV infection, and its expression on effector cells may mark those cells destined to die (24, 25). What role this marker plays on memory cells is unclear, although influenza memory cells expressed significantly lower levels of KLRG-1 in all tissues examined, as they differentiated from naive to effector to memory cells.

Granzyne B expression by effector cells after LCMV or influenza virus infection correlated with virus levels in the tissues (Fig. 2). Responder cells in the spleen produced significantly more granzyme B after LCMV infection, corresponding with the presence of virus. Similar levels of granzyme B were observed in the lungs of mice infected with either virus; however, specific CD8+ T cells remained granzyme B+ for ≥1 wk longer after PR8-33 infection. Similarly, the high m.w. form of CD43 recognized by the 1B11 Ab remained significantly higher on memory gp33-specific cells in the lung after PR8-33 infection, possibly reflecting the duration of Ag exposure and killing function by CTLs. This is consistent with papers showing the prolonged presence of influenza virus Ags in the lung postinfection (26, 27).

**Reduced cytokine production by memory CD8+ T cells after influenza virus infection**

The proportion of Ag-specific cells producing the cytokines IFN-γ, TNF-α, and IL-2 was similar after LCMV or PR8-33 infection (Fig. 3A, 3B), although we consistently observed slightly fewer IL-2–expressing memory P14 cells in the spleen, LNs, and lungs after PR8-33 infection in comparison with memory cells generated after LCMV infection (Fig. 3B). We next sought to examine, in more detail, cytokine production by memory CD8+ T cells generated after influenza virus and LCMV infections. To more closely examine cytokine production, we stimulated memory cells from LCMV- or PR8-33–immune mice with decreasing concentrations of gp33 peptide (10−7–10−12 M) and measured intracellular cytokine accumulation after 5 h. The proportion of LCMV or PR8-33 memory cells producing IFN-γ or TNF-α was identical over the range of peptide concentrations tested, although consistently fewer PR8-33 memory cells produced IL-2 (LCMV: 31.4 ± 1.7%; PR8-33: 20.2 ± 5.7% at 10−8 M) (Fig. 3C). Importantly, mean levels of expression of IFN-γ, TNF-α, and IL-2 were significantly lower in PR8-33 memory cells compared with LCMV memory cells (Fig. 3D). Normalization of the curves to reflect the percentage of the maximum cytokine production by LCMV or PR8-33 memory cells demonstrated similar responsiveness to different concentrations of Ag (Fig. 3E). Thus, the PR8-33 memory cells did not display a reduced sensitivity to decreasing concentrations of Ag compared with LCMV memory cells, yet PR8-33 memory cells produced slightly lower levels of cytokines on a per-cell basis upon stimulation with all doses of peptide.

**Reduced proliferative responses to Ag or homeostatic cytokines by influenza memory cells**

We next examined the proliferative capacity of the influenza memory cells in response to homeostatic cytokines or Ag. Memory CD8+ T cells generated after LCMV or PR8-33 infection expressed similar levels of TCR, the common γ-chain (CD132), IL-2/IL-15Rβ (CD122), IL-7Rα (CD127), and IL-15Rα (Fig. 4A). Despite similar surface expression of these cytokine receptors, PR8-33 memory cells demonstrated reduced proliferation in response to IL-2, IL-7, and IL-15 compared with LCMV memory cells (Fig.
Both memory populations proliferated equally well upon stimulation with $10^{-7}$ M gp33 peptide; however, PR8-33 memory cells displayed reduced responsiveness upon stimulation with lower concentrations of peptide ($10^{-10}$ M) that approach a more physiologic range (Fig. 4C). Thus, PR8-33 memory cells demonstrated reduced responsiveness to common $\gamma$-chain cytokines and Ag in vitro compared with LCMV immune memory CD8+ T cells.

Recall responses and protective immunity by influenza or LCMV memory cells

Given the qualitative differences between memory cells primed after LCMV or influenza virus infection that we observed ex vivo, we next wanted to evaluate the protective capacity of memory CD8+ T cells generated after PR8-33 versus LCMV infection in vivo. Significant numbers of memory cells could be detected in the spleen, lungs, LNs, and airways after LCMV and PR8-33 infection (Figs. 1, 5A, data not shown). In all tissues, with the exception of the airways, greater numbers of gp33-specific cells were found after LCMV infection. We transferred normalized numbers of Thy1.1+ CD8+ memory T cells (1–5 × 10^4), isolated from the spleen or lungs, into naive mice and the next day challenged them intranasally with a recombinant vaccinia virus expressing the gp33 epitope (VV-33) (Fig. 5C). The transferred memory cells were phenotypically similar, with slightly higher KLRG-1 expression on a proportion of LCMV memory cells and 1B11 expression on a proportion of lung-derived PR8-33 memory cells (Fig. 5B).
Expansion of spleen- or lung-derived memory cells in the blood after VV-33 infection peaked around day 15 postinfection (Fig. 5D). Interestingly, spleen- or lung-derived memory cells from PR8-33 immune mice expanded considerably less than did those from LCMV immune mice (∼2- or 7-fold less, respectively, at day 15). Examination of the responding Thy1.1+ CD8+ memory T cells in the tissues 8 d postinfection revealed similar differences in cellular expansion in the spleen, lungs, and airways (BAL) (Fig. 5E). Most importantly, viral control in the lungs 4 d postinfection was significantly reduced after transfer of PR8-33 memory cells compared with LCMV memory cells (Fig. 5E). Thus, spleen- and lung-derived LCMV memory CD8+ T cells expanded and controlled virus more effectively than did influenza memory cells following secondary infection.

Because differences in precursor cell frequency can potentially influence the size and phenotype of the resulting memory CD8+ T cells, we compared memory T cell generation after the transfer of a low number of naive P14 CD8+ T cells (10^3) into mice, followed by LCMV or PR8-33 infection. Substantial numbers of memory cells could be detected in the spleen and lungs 3 mo after LCMV and PR8-33 infection (data not shown). The memory CD8+ T cells expressed a similar phenotype to those primed from a greater precursor frequency. This included higher 1B11 expression on PR8-33 memory cells, as well as higher KLRG1 and granzyme B expression on LCMV memory cells.

Importantly, memory cells primed from a lower precursor frequency also displayed reduced cytokine production after PR8-33 infection compared with LCMV-primed memory cells (data not shown), mirroring that observed when the cells were primed at a higher precursor frequency (Fig. 3C–E). Lastly, in recall experiments, PR8-33 lung memory cells primed from a low precursor frequency expanded significantly less than did LCMV-primed memory cells, demonstrating that qualitatively different memory CD8+ T cells were generated after systemic LCMV infection and respiratory influenza virus infection.

Discussion

Despite significant advances in our understanding of the signals required for the priming of memory T cells, there is still much to understand with regard to the roles of different tissues in influencing
the type of memory that is generated. To better understand the differences between T cells primed by mucosal or systemic viral infection we compared CD8+ T cells generated after influenza and LCMV infections. We demonstrate that influenza virus infection generated functionally different memory cells compared with LCMV infection, resulting in suboptimal recall responses upon secondary challenge. Memory CD8+ T cells from influenza virus-immune mice displayed a reduced proliferative capacity in vivo upon recall, as well as in vitro in the presence of peptide or common γ-chain cytokines, compared with LCMV memory cells. Influenza and LCMV memory cells displayed equivalent sensitivity to different concentrations of Ag in vitro, as measured by the ability to produce cytokines. Nevertheless, influenza memory cells produced lower levels of effector cytokines at all doses of Ag, suggesting an intrinsic functional difference from memory cells primed after LCMV infection.

During the priming phase of an immune response, factors, including the amount of available Ag, the duration and strength of TCR signaling and costimulation, CD4+ T cell help, inflammation, and the availability of cytokines, are integral to the size and quality of the resulting effector and memory T cell pool (7). Differences in viral tropism and replication during priming or immunization with nonreplicating Ags or vaccine vectors that induce low levels of Ag and inflammation can greatly influence the quality of the resulting memory pool (30). Despite this knowledge, it is much less clear how systemic or mucosal infections differ in the priming of T cell responses. A number of reports showed that LNs draining mucosal sites, such as the gut, can prime T cells with the capacity to preferentially home to mucosal sites (31). Similar imprinting has not been shown for respiratory infections. Moreover, systemic infection can prime cells capable of migrating to all regions of the body (8, 9). However there has been little comparison of the qualitative differences between memory cells primed by systemic versus mucosal infections. In this study, we examined this and found qualitative differences between LCMV and influenza virus infections resulting in functional differences in recall and protective capacity.

It is tempting to speculate whether the programming of memory CD8+ T cells after influenza infection was suboptimal. That the PR8-33 cells expressed lower levels of the cytokines IFN-γ, TNF-α, and IL-2 and expressed less KLRG-1 may be indicative of a less-activated phenotype (24). We also found that CD8+ T cells in the lung expressed more CD43 and granzyme B, seeming to be activated for longer after PR8-33 infection (Fig. 2). This may be due to the prolonged presence of Ag after influenza virus infection (26, 27). It is unclear whether such persistent, low-level presentation of Ag after influenza virus infection may influence the functional quality of the memory cells generated. Such differences in memory T cell function may also be influenced by changes in gene expression that is controlled by epigenetic changes that occur in T cells after activation (32). Finally, although we did not separate memory cells into effector and central memory subsets, the majority of cells from LCMV- and influenza immune mice were CD62L+, or central memory phenotype, after 2–3 mo. Thus, our data indicate that memory cell heterogeneity cannot be predicted entirely on the basis of surface marker expression.

These data suggest that the potential for influenza vaccines that elicit heterosubtypic immunity through conserved or cross-reactive epitopes may be influenced by the quality of the memory T cells generated. It is thought that a strong correlate of protection from respiratory infection is the presence of effector or memory T lymphocytes in the airways (33). We showed in this study that influenza-primed memory cells expanded less vigorously than did LCMV-derived memory cells in the lungs after a secondary respiratory challenge, resulting in slightly delayed viral clearance. It is possible that memory cells may not need to expand as rapidly following influenza infection, because pre-existing cells in the lungs of intact animals should provide a short-term control of viral growth while activation of central memory cells occurs in the draining LNs (34). However, a reduction in the number of Ag-specific cells occurs in the airways in the first few months post-infection (35, 36), possibly due to decreased recruitment into the airways (37). This would suggest that a vigorous response upon reinfection would be of benefit to the host. Following recall with VV-33, PR8-33 memory cells expanded less than did LCMV memory cells and demonstrated reduced killing of peptide-labeled targets in an in vivo cytotoxicity assay 5 d after VV-33 infection (S.N. Mueller and R. Ahmed, unpublished observations).
It is unclear to what extent expansion of the memory CD8+ T cells versus cytokine production or cytotoxicity influenced viral clearance upon VV-33 recall. However, these processes are not necessarily separable because reduced expansion, cytokine production, and responsiveness to homeostatic cytokines by the PR8-33 memory cells may be influenced by similar downstream signaling events. Nevertheless, rapid and robust expansion of memory T cells is important for protection against reinfection, and suboptimal expansion likely affects the outcome of disease.

It is feasible that influenza-primed memory cells continue to differentiate and, over time, gain an improved ability to proliferate upon reinfection (38). However, these processes are not necessarily separable because reduced expansion, cytokine production, and responsiveness to homeostatic cytokines by the PR8-33 memory cells may be influenced by similar downstream signaling events. Nevertheless, rapid and robust expansion of memory T cells is important for protection against reinfection, and suboptimal expansion likely affects the outcome of disease.

Infection of C57BL/6 mice with the A/PR/8/34 strain of influenza results in a robust respiratory infection marked by high viral titers, weight loss, and a significant adaptive immune response (42). Influenza virus encodes the nonstructural protein NS1, which has multiple functions, not the least of which is the ability to bind dsRNA and viral RNA and reduce type I IFN production and impact on genes in the IFN pathway (43–45). Given the role of IFN-α/β in promoting CD8+ and CD4+ T cell responses (46–48), NS1-mediated restriction of IFN-α/β production during influenza virus infection may also affect optimal CD8+ T cell priming. We showed that recombinant influenza viruses lacking expression of the NS1 protein or with truncated NS1 proteins prime fully functional memory CD8+ T cells (49). These memory T cells responded slightly better upon recall infection than did memory stimulation suggest that the phenotype and function of memory cells can be tuned over time. Thus, it will be interesting to determine whether the reduced functionality of memory CD8+ T cells primed after influenza virus infection in our system can be altered following secondary or even tertiary stimulation.

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T cells primed by wild-type influenza virus, suggesting a possible role for influenza NS1 proteins in influencing T cell responses and memory postinfection. It is clear that considerable heterogeneity exists within memory T cell pools, although we are only just beginning to appreciate this functional heterogeneity, with a far from complete understanding as to how complex arrays of signals can program such diversity. We showed in this article that two robust viral infections can generate functionally different memory T cells. It is unclear whether reduced memory CD8+ T cell functionality is unique to influenza virus infection or whether other respiratory infections might also prime memory T cells with similar qualities. It will be interesting to determine whether infections that remain localized to tissues (including the respiratory tract, intestines, and skin) can prime memory T cells with different functionality compared with those primed following systemic infection. Our data may provide insight into the efficacy of cross-reactive T cell memory in protection against heterologous virus infections and understanding how to trigger long-lasting protective immune responses.

Disclosures
The authors have no financial conflicts of interest.

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