Attrition of memory CD8 T cells

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An important role for the immune system is to maintain protective immunological memory to a wide variety of pathogens encountered over one's lifetime, while still leaving the host able to respond to newly encountered pathogens. Vezys *et al.*[1] make the interesting observation that it is possible to repeatedly immunize mice in ways that allow for development of high numbers of memory CD8 T cells without depleting pre-existing memory cells specific for other pathogens. This study, which offers promise in developing potent vaccination schemes, is seemingly at odds with work published by us in the 1990s showing a loss in CD8 memory cells after a series of infections[2,3]. In their reply, Vezys *et al.* mention that we may have misinterpreted our data because we reported the putative loss of memory T cells as per cent rather than total number, but here we represent the data in those studies as total cell number[2–4]. We show here in Fig. 1 that a series of infections can indeed reduce the total number of memory cells, indicating that vaccination strategies need to consider this issue.

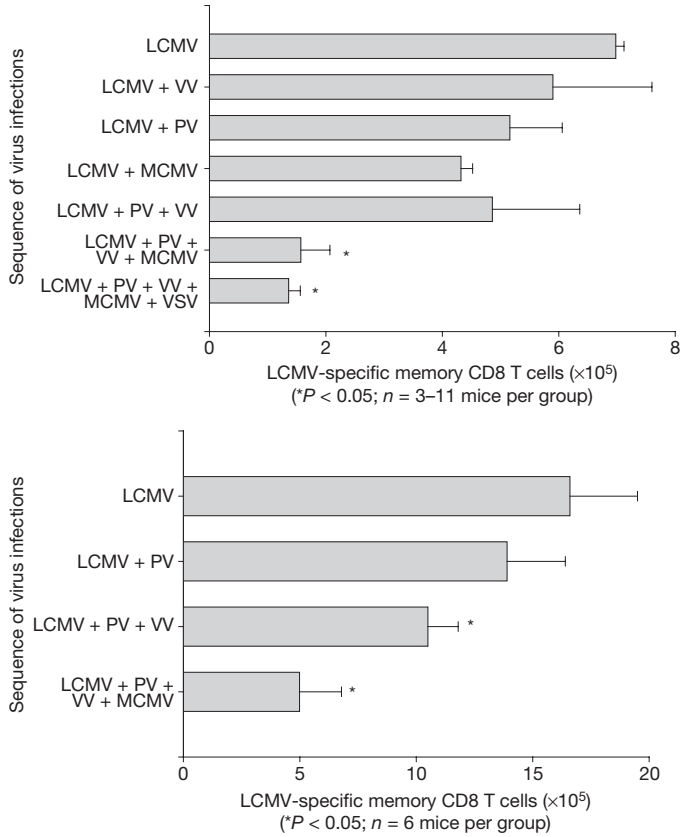

Figure 1 | Depletion of memory CD8 T cells by heterologous viral infections. Total numbers of lymphocytic choriomeningitis virus (LCMV)-epitope-specific CD8 T cells per spleen in mice immune to LCMV and subsequently infected with Pichinde virus (PV), vaccinia virus (VV), murine cytomegalovirus (MCMV) or vesicular stomatitis virus (VSV). Data are reworked from refs 3 and 4. These are controlled experiments in which all measurements were taken at the same time after LCMV infection. **a,** Attrition of LCMV-specific memory cells as measured by limiting dilution assays after sequential virus infections. **b,** Attrition of LCMV-specific memory cells as measured by MHC-dimer or intracellular IFN-γ assays after sequential virus infections. Combined frequencies of T cells specific to LCMV-encoded epitopes NP396, GP33/34 and GP276 (GP, glycoprotein; NP, nucleoprotein).

Figure 1a portrays limiting dilution assay data, a measure mostly of central memory cells, with 3 to 11 determinations per group, and Fig. 1b shows determinations with intracellular cytokine and MHC-dimer assays, with 6 determinations per group. They show that losses in memory CD8 cells became statistically significant after a series of viral infections. Virus-induced losses in total body memory have also been reported by *in vivo* cytotoxicity assays, which monitor the ability of T cells to kill antigen-expressing targets in the host[5]. Others have reported bacteria-induced losses of T-cell-dependent immunity to tumours[6]. These findings differ with the conclusions of Vezys *et al.*[1], who examined loss of memory cells after infections with only a single pathogen or after a series of immunizations with vectors encoding the same antigen in a prime–boost immunization scheme.

The relevance of that point rests on the observation that virus-induced type 1 interferon (IFN) mediates high levels of apoptosis of memory T cells early during infections[7–10]. We previously addressed the question of whether the long-term loss of memory cells was a function of this early apoptosis (active attrition) or due to a competition model for niches in the immune system (passive attrition), and concluded that most of the loss in memory cells could be accounted for by active attrition rather than competition[5]. We question whether the prime–boost immunization scheme of Vezys *et al.*[1] would elicit the apoptosis-inducing levels of type 1 IFN that a series of different viruses would, because the homologous immunity elicited to their immunizing antigen inserts would inhibit replication of the vectors. Furthermore, recombinant vaccinia virus like that used in this study is a poor IFN inducer. A recent paper[11] reports a substantial type 2 IFN (IFN-γ)-dependent permanent loss of memory CD8 T cells in mice challenged with intracellular bacteria that were strong IFN-γ (but not type 1 IFN) inducers. There it was also concluded[11] that the loss of memory cells was due to an active process and not due to competition. This indicates that more than one class of IFN can cause memory T-cell attrition.

Vezys *et al.*[1] state that an argument for memory CD8 T-cell stability is a human study showing a half-life of 8–16 years for vaccinia-virus-specific CD8 T cells[12]. However, that same study could not detect vaccinia-virus-specific CD8 T cells in 50% of the subjects vaccinated over 20 years earlier. We contend that those data are more consistent with a loss in human memory T cells than of memory cell stability.

We therefore argue that the study of Vezys *et al.*[1] does not refute earlier work showing that a series of infections depletes splenic memory CD8 cells specific to previously encountered pathogens. It does, however, nicely show how one can avoid such depletion by using a prime–boost immunization scheme with vectors that are not strong IFN inducers.

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1. Vezys, V. *et al.* Memory CD8 T-cell compartment grows in size with immunological experience. *Nature* **457,** 196–199 (2009).
2. Selin, L. K., Vergilis, K., Welsh, R. M. & Nahill, S. R. Reduction of otherwise remarkably stable virus-specific cytotoxic T lymphocyte memory by heterologous viral infections. *J. Exp. Med.* **183,** 2489–2499 (1996).
3. Selin, L. K. *et al.* Attrition of T cell memory:selective loss of lymphocytic choriomeningitis virus (LCMV) epitope-specific memory CD8 T cells following infections with heterologous viruses. *Immunity* **11,** 733–742 (1999).
4. Varga, S. M., Selin, L. K. & Welsh, R. M. Independent regulation of lymphocytic choriomeningitis virus-specific T cell memory pools: relative stability of CD4 memory under conditions of CD8 memory T cell loss. *J. Immunol.* **166,** 1554–1561 (2001).
5. Kim, S. K. & Welsh, R. M. Comprehensive early and lasting loss of memory CD8 T cells and functional memory during acute and persistent viral infections. *J. Immunol.* **172,** 3139–3150 (2004).

6. Smith, D. K. *et al.* Cross-reactive antigen is required to prevent erosion of established T cell memory and tumor immunity: a heterologous bacterial model of attrition. *J. Immunol.* **169,** 1197–1206 (2002).
7. McNally, J. M. *et al.* Attrition of bystander CD8 T cells during virus-induced T cell and interferon responses. *J. Virol.* **75,** 5965–5976 (2001).
8. Jiang, J., Lau, L. L. & Shen, H. Selective depletion of nonspecific T cells during the early stage of immune responses to infection. *J. Immunol.* **171,** 4352–4358 (2003).
9. Bahl, K. *et al.* Interferon-induced attrition of CD8 T cells in the presence or absence of cognate antigen during the early stages of viral infections. *J. Immunol.* **176,** 4284–4295 (2006).
10. Jiang, J., Gross, D., Nogusa, S., Elbaum, P. & Murasko, D. M. Depletion of T cells by type I interferon: differences between young and aged mice. *J. Immunol.* **175,** 1820–1826 (2005).
11. Dudani, R., Murali-Krishna, K., Krishnan, L. & Sad, S. IFN-γ induces the erosion of preexisting CD8 T cell memory during infection with a heterologous intracellular bacterium. *J. Immunol.* **181,** 1700–1709 (2008).
12. Hammarlund, E. *et al.* Duration of antiviral immunity after smallpox vaccination. *Nature Med.* **9,** 1131–1137 (2003).

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Vezys et al. *reply*

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We reported that it is possible to increase the total number of memory CD8 T cells within an organism, and to establish preternatural numbers of vaccine-specific effector memory CD8 T cells while preserving naive CD8 T cells and most pre-existing memory CD8 T cells specific for a previously encountered infection[1]. These findings raise new questions regarding the regulation and limits of generating CD8 T cell immunity. Our discussion highlighted three points related to the issue of attrition. First, that it is possible to over-estimate perceived attrition by only examining percentages (see Fig. 1 of ref. 1)[2]. Second, our vaccine regimen resulted predominantly in the generation of effector memory CD8 T cells located outside of lymph nodes. It remains possible that the number of lymph node central memory T cells remains tightly regulated. Third, we noted that our data did not refute that attrition could happen under a variety of circumstances. However, our data demonstrate that attrition is not an axiomatic property of immunization, mandated by stringent regulation of the size of the total memory CD8 T-cell compartment. Indeed, we saw no evidence of attrition after single infections with a virus (vaccinia), an intracellular bacteria (*Listeria monocytogenes*) and a parasite that induces massive splenomegaly (*Plasmodium yoelii*), and observed comparatively little attrition after a heterologous prime–boost regimen involving successive immunization with three viruses[1].

The issue at hand is whether our results represent rare exceptions that could be exploited for vaccination, or whether preservation of immunological memory is a normal feature of the mammalian immune system. Welsh and Selin[2] argue that memory T-cell maintenance is regulated by the quantity of type 1 IFN produced in response to subsequent infections, and that most infections invariably lead to profound loss of pre-existing memory CD8 T cells. Data supporting this claim demonstrate that up to 25–90% of pre-existing memory CD8 T cells are lost after single infections, and that attrition accumulates further with each infection[3,4]. Surprisingly, infection-specific naive and memory T cells were not spared[5]. The current model of Welsh and Selin[2] predicts that immunological memory must be short-lived in the face of occasional infections. Consequently, they question the evidence that CD8 T-cell memory specific for vaccinia has a long half-life in humans[6]. Memory CD8 T cells have also been examined after a single exposure to both measles and hantavirus, and robust populations could be detected >10 years later[7,8]. Protective immunity against measles virus (for which T-cell memory may be important) persists >65 years (ref. 9). Memory B cells persist indefinitely after smallpox immunization, and the half-life of serum antibody, which must be continuously produced by differentiated antigen-experienced B cells, ranges from 92 to 3,014 years after exposure to measles, mumps, rubella or vaccinia virus[10,11]. These data indicate that immunological memory does not necessarily undergo rapid erosion, although further studies regarding the longevity of CD8 T memory are needed.

The concept advanced by our paper is that attrition is not an axiomatic property of immunization, and that the memory CD8 T-cell compartment is capable of expansion. In support of these findings, it was recently reported that primary human cytomegalovirus infection resulted in a long-lived increase in the total number of antigen-experienced CD8 T cells, and induced a reduction in the frequency, but not number, of pre-existing memory CD8 T cells specific for influenza or Epstein–Barr viruses in blood[12]. It should be stressed that immunity to certain agents (for example, after vaccination with non-replicating agents) may be intrinsically prone to attrition, and certain infections may significantly erode immunological memory. These may include pathogens that infect lymphocytes, destroy lymphoid tissue or are associated with other pathologies, as indicated by Welsh and Selin[2]. Although our results raise the bar for what levels of CD8 T-cell memory might be achievable through vaccination, Welsh and Selin[2] highlight the important fact that each vaccine vector should be evaluated empirically for safety and the preservation of pre-existing immunological memory.

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1. Vezys, V. *et al.* Memory CD8 T-cell compartment grows in size with immunological experience. *Nature* **457,** 196–199 (2009).
2. Welsh, R. M. & Selin, L. K. Attrition of memory CD8 T cells. *Nature* **459,** doi:10.1038/nature08091 (2009).
3. Dudani, R., Murali-Krishna, K., Krishnan, L. & Sad, S. IFN-γ induces the erosion of preexisting CD8 T cell memory during infection with a heterologous intracellular bacterium. *J. Immunol.* **181,** 1700–1709 (2008).
4. Selin, L. K. *et al.* Attrition of T cell memory: selective loss of LCMV epitope-specific memory CD8 T cells following infections with heterologous viruses. *Immunity* **11,** 733–742 (1999).
5. Bahl, K. *et al.* IFN-induced attrition of CD8 T cells in the presence or absence of cognate antigen during the early stages of viral infections. *J. Immunol.* **176,** 4284–4295 (2006).
6. Hammarlund, E. *et al.* Duration of antiviral immunity after smallpox vaccination. *Nature Med.* **9,** 1131–1137 (2003).
7. Van Epps, H. L. *et al.* Long-lived memory T lymphocyte responses after hantavirus infection. *J. Exp. Med.* **196,** 579–588 (2002).
8. Nanan, R., Rauch, A., Kampgen, E., Niewiesk, S. & Kreth, H. W. A novel sensitive approach for frequency analysis of measles virus-specific memory T-lymphocytes in healthy adults with a childhood history of natural measles. *J. Gen. Virol.* **81,** 1313–1319 (2000).
9. Panum, P. L. *Observations Made During the Epidemic of Measles on the Faroe Islands in the Year 1846* (Delta Omega Society, 1940).
10. Crotty, S. *et al.* Cutting edge: long-term B cell memory in humans after smallpox vaccination. *J. Immunol.* **171,** 4969–4973 (2003).
11. Amanna, I. J., Carlson, N. E. & Slifka, M. K. Duration of humoral immunity to common viral and vaccine antigens. *N. Engl. J. Med.* **357,** 1903–1915 (2007).
12. van Leeuwen, E. M. *et al.* Differential usage of cellular niches by cytomegalovirus versus EBV- and influenza virus-specific $CD8^+$ T cells. *J. Immunol.* **177,** 4998–5005 (2006).

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