ivy rash this spring, they should know that NK cells also seem to mediate CHS.

1. O'Leary, J.G., Goodarzi, M., Drayton, D.L. & von Andrian, U.H. *Nat. Immunol.* **7**, 507–516 (2006).
2. Belsito, D.V. Allergic contact dermatitis. In *Fitzpatrick's Dermatology in General Medicine* 5th edn. (eds. Freedberg, I.M. *et al.*) 1447–1461 (McGraw-Hill, New York, 1999).
3. Janeway, C.A., Jr., Travers, P., Walport, M. & Shlomchik, M. *Immunobiology. The Immune System in Health and Disease* 5th edn. (Garland, New York, 2001).
4. Yokoyama, W.M. Natural killer cells. In *Fundamental Immunology* 4th edn. (ed. Paul, W.E.) Ch. 17, 575–603 (Lippincott-Raven, New York, 1999).
5. Dokun, A.O. *et al. Nat. Immunol.* **2**, 951–956 (2001).
6. Martin-Fontecha, A. *et al. Nat. Immunol.* **5**, 1260–1265 (2004).
7. Koka, R. *et al. J. Exp. Med.* **197**, 977–984 (2003).
8. Bauer, S. *et al. Science* **285**, 727–729 (1999).
9. Grabbe, S. *et al. J. Clin. Invest.* **98**, 1158–1164 (1996).
10. Wu, J. *et al. Science* **285**, 730–732 (1999).
11. Diefenbach, A. *et al. Nat. Immunol.* **3**, 1142–1149 (2002).
12. Kim, S. *et al. Nature* **436**, 709–713 (2005).

On the TRAIL of homeostatic memory T cells

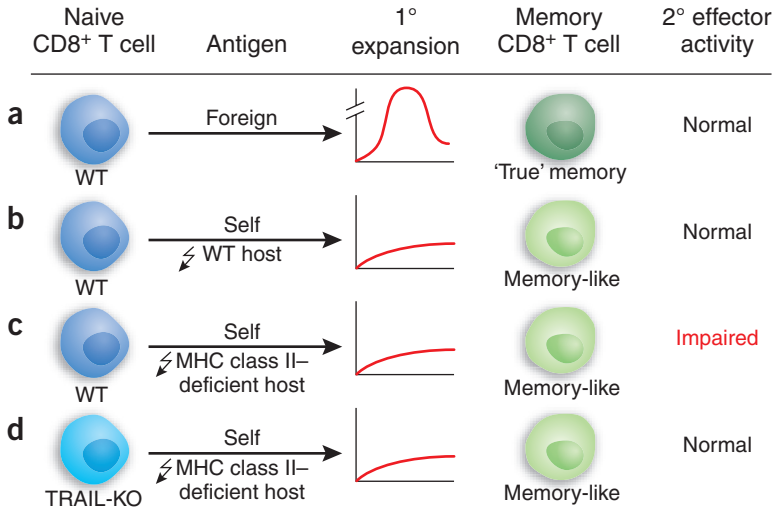
Charles D Surh & Jonathan Sprent

The resolution of immune responses typically leaves a population of memory T cells to respond to subsequent infection. The generation of 'memory-like' T cells can also occur during homeostatic proliferation, but are they 'true' memory cells?

It is well established that maintenance of antigen-specific memory T cells confers long-term resistance to subsequent infection by the same pathogen. Studies have also demonstrated that 'memory-like' $CD4^+$ and $CD8^+$ T cells, with features similar to those of antigen-specific memory cells, can arise spontaneously from naive T cells placed in an environment (often lymphopenic) containing large amounts of the endogenous factors that normally govern T cell survival and homeostasis. In this issue of *Nature Immunology*, Jameson and colleagues demonstrate that memory-like $CD8^+$ T cells formed during so-called 'homeostatic proliferation' closely resemble antigen-specific memory $CD8^+$ T cells both in the capacity to confer effector protection after secondary challenge with *Listeria monocytogenes* (*L. monocytogenes*) and in the dependence on $CD4^+$ T cell help to become effector cells[1].

In a typical immune response to a new infection, naive $CD8^+$ T cells specific for the pathogen are induced to undergo massive population expansion and to acquire the functional capabilities necessary to resolve the infection[2]. Once activated, such cells divide very rapidly (every 6–8 h) for 4–5 d while differentiating into effector cells that are able to kill antigen-presenting and infected cells and release cytokines, such as interferon-γ and tumor necrosis factor, thus leading to rapid elimination of the pathogen. When the immune response resolves, numerous $CD8^+$ effector T cells are no longer needed and so most die by apoptosis. However, a small fraction of effector cells survives as long-lived memory cells that can respond swiftly to the same pathogen if encountered again.

In contrast, 'memory-like' $CD8^+$ T cells produced during homeostatic proliferation arise only when naive $CD8^+$ T cells find lymphopenic environments, such as in irradiated wild-type mice or in mice deficient in recombination-activating gene expression. In such contexts, naive



Katie Ris

Figure 1 The protective capacity of memory-phenotype $CD8^+$ T cells generated in response to lymphopenia requires $CD4^+$ T cell help. (**a**,**b**) In addition to the normal mechanism of memory $CD8^+$ T cell production during responses to foreign antigens (**a**), 'memory-like' cells can arise spontaneously when naive $CD8^+$ T cells are exposed to self antigens in lymphopenic conditions (**b**). In the latter situation, naive $CD8^+$ T cells progress directly to the memory stage through a continuous process of slow 'homeostatic' population expansion, thus bypassing the phases of considerable population expansion, death and effector cell differentiation that typically occur for antigen-specific T cells. (**c**) Memory-like $CD8^+$ T cells closely resemble true memory $CD8^+$ T cells in their ability to mediate vigorous secondary immune response and in their requirement for $CD4^+$ T cell help during development. As with memory T cells, TRAIL is the key negative regulator of secondary responses by memory-like $CD8^+$ T cells generated in the absence of $CD4^+$ T cell help, although the TRAIL-dependent mechanism may differ for the two memory cell types. (**d**) Unlike normal cells, TRAIL-deficient $CD8^+$ T cells can develop into fully functional memory-like cells in the absence of $CD4^+$ T cell help. WT, wild-type; TRAIL-KO, TRAIL deficient; lightning symbol, irradiation.

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CD8$^+$ T cells undergo slow homeostatic proliferation while acquiring phenotypic and functional characteristics of antigen-specific memory CD8$^+$ T cells, including the capacity to secrete interferon-γ, and demonstrate cytotoxic activity after stimulation with the cognate antigen[3,4]. As indicated by the name, 'homeostatic proliferation' is driven by endogenous homeostatic factors. In most cases these factors include (at least) self major histocompatibility complex (MHC)–peptide and a constitutively produced 'homeostatic' cytokine interleukin 7 (IL-7), which in normal physiological conditions is vital in keeping both naive and memory T cells alive in a resting state[5]. Lymphopenia-driven homeostatic proliferation probably occurs when IL-7 increases above a certain threshold; because IL-7 is an effective costimulator, the increased IL-7 presumably intensifies the weak T cell receptor signaling delivered by self MHC–peptide, thus inducing naive T cells to enter the cell cycle. Compared with antigen-driven proliferation, homeostatic proliferation is very slow, with a cycling time of 24–36 h, and leads to a gradual accumulation of memory-like cells that resemble antigen-specific memory cells despite the absence of stimulation by foreign MHC–peptide and the tumultuous episodes of overt activation, massive population expansion and severe population contraction associated with antigen-specific memory T cell production.

To test the functional capability of 'memory-like' CD8$^+$ T cells generated via homeostatic proliferation ('HP-memory cells'), Hamilton *et al.* adoptively transferred ovalbumin (OVA)–specific T cell receptor–transgenic OT-I CD8$^+$ T cells ('OT-I cells') into syngeneic, lightly irradiated wild-type hosts and then waited 3 weeks, allowing the cells to divide. The donor OT-I cells were then collected and tested for their capacity to protect secondary wild-type recipient hosts against challenge with high-dose *L. monocytogenes* engineered to express OVA (LM-OVA). As predicted from published reports, HP-memory OT-I cells are much better than naive OT-I cells and are just as effective as 'true' memory OT-I cells (that is, those produced after challenge with antigen and resolution of the immune response) in protecting the hosts from *L. monocytogenes* infection. HP-memory OT-I cells also closely resemble true memory cells in their ability to undergo vigorous secondary population expansion and to demonstrate effector function on a per-cell basis. Given those results, the authors then tested if the resemblance between the 'two types' of memory cells also includes the requirement for CD4$^+$ T cell 'help' during their development.

Although CD4$^+$ T cells are not required for the development of efficient primary CD8$^+$ T cell responses to foreign antigens, published work has shown that without CD4$^+$ T cells, memory CD8$^+$ T cells have a shortened lifespan and produce poor secondary cell population expansion and effector generation[6–9]. Why CD4$^+$ T cell help is required for these functions is controversial, but it seems to center on a temporal requirement by developing memory CD8$^+$ T cells for help. Some argue that CD4$^+$ T cells are essential only during the initial priming of naive CD8$^+$ T cells to differentiate into functional memory cells[6–8]. However, others maintain that CD4$^+$ T cells are crucial only after the formation of resting memory CD8$^+$ T cells (that is, well after the primary response has ended)[10]. The debate on when and how CD4$^+$ T cells 'help' CD8$^+$ T cells has yet to be resolved, although evidence suggests that a defect in memory CD8$^+$ T cells that develop in the absence of CD4$^+$ T cells is mediated by TRAIL (tumor necrosis factor–related apoptosis-inducing ligand)[11]. Expression of TRAIL, a secreted proapoptotic molecule, is upregulated only in activated memory CD8$^+$ T cells that develop in the absence of CD4$^+$ T cell help. The mechanism that has been proposed is that expression of TRAIL causes death ('fratricide') of other T cells, as the TRAIL receptor is upregulated on effector cells. In support of that idea, abrogation of TRAIL signaling restores the functional capabilities of memory CD8$^+$ T cells that develop in the absence of CD4$^+$ T cell help, whereas the presence of either exogenous TRAIL or CD8$^+$ T cells that develop in the absence of CD4$^+$ T cell help inhibits the function of antigen-specific (true) memory CD8$^+$ T cells.

Hamilton *et al.* have now demonstrated that HP-memory CD8$^+$ T cells are similar to true memory CD8$^+$ T cells in their requirement for CD4$^+$ T cell help[1] (**Fig. 1**). First, they show that HP-memory OT-I cells generated in the absence of CD4$^+$ T cell help (OT-I cells transferred into irradiated MHC class II–deficient hosts) are no better than naive OT-I cells in clearing wild-type hosts after challenge with LM-OVA. The reduced effector function of the HP-memory CD8$^+$ T cells reflects a functional deficit due to the absence of CD4$^+$ T cells rather than MHC class II molecules themselves, as a similar defect is found when naive OT-I cells are transferred to congenitally T cell–deficient (T cell receptor-α–deficient and recombination-activating gene–deficient) hosts. It is also notable that because the wild-type recipients of the OT-I cells were never exposed to OVA, the generation of fully functional HP-memory CD8$^+$ T cells does not require antigen-specific CD4$^+$ T cells. The authors also show that as with true memory CD8$^+$ T cells, expression of TRAIL by HP-memory CD8 cells generated in the absence of CD4 T cell help is the eventual cause of their reduced function. When TRAIL-deficient OT-I cells are transferred to MHC class II–deficient hosts, the HP-memory cells generated have normal recall responses and efficiently clear LM-OVA infection of wild-type hosts. Although the issue of when CD4$^+$ T cell help is required for HP-memory CD8$^+$ T cells (during or after priming) is not addressed directly, the function of HP-memory cells generated in MHC class II–deficient hosts is found to decrease progressively over time, suggesting that help is crucial for maintaining long-term HP-memory CD8$^+$ T cell survival.

Despite their similarity, HP-memory and true memory CD8$^+$ T cells have subtle functional differences in their requirement for CD4$^+$ T cell help. Whereas true memory CD8$^+$ T cells generated without help are severely impaired in their ability to undergo population expansion and effector generation[7–9], HP-memory CD8$^+$ T cells generated without help show only a slight delay in their rate of population expansion and mediate near-normal effector function, such as cytokine production and cytotoxic activity. Another key difference is that although true memory CD8$^+$ T cells generated without help can inhibit normal memory responses by 'paralyzing' bystander memory cells via expression of TRAIL, HP-memory CD8$^+$ T cells generated without help do not apparently use the same TRAIL-dependent mechanism even though they express TRAIL.

It seems unlikely that a slight delay in the rate of secondary population expansion could render HP-memory CD8$^+$ T cells generated without help incapable of protecting hosts from LM-OVA infection. Instead, the variability in the activity of HP-memory and true memory OT-I cells generated without help may reflect the possibility that these two types of cells are 'wired' differently, especially in the production of and responsiveness to TRAIL. TRAIL and its receptor are readily induced in true

memory cells generated without help and begin to cause TRAIL-dependent cell death soon after antigen stimulation[11]. However, in contrast to the mechanism proposed for true memory CD8$^+$ T cells, TRAIL produced by HP-memory cells does not seem function by inducing cell death. One possibility for the difference is that expression of TRAIL and its receptor are delayed in HP-memory cells generated without help (and are not expressed at all for helped cells), thereby limiting the effects of TRAIL on the suppression of terminal effector function. In addition, it is possible that HP-memory and true memory CD8$^+$ T cells generated without help may differ in the way they interact with other cells, such as dendritic cells and macrophages, that participate in the clearance of *L. monocytogenes*.

Whatever the explanation, the important findings are that functionally competent memory CD8$^+$ T cells can be generated through homeostatic proliferation without exposure to specific antigens and that these cells closely resemble true memory cells in their requirement for CD4$^+$ T cell help. Given that memory-like cells are abundant in normal animals and humans, as well as in animals raised in germ-free conditions[12], a key issue is whether these cells can provide added protection in responses to new pathogens beyond that conferred by naive T cells. Memory-like T cells are known to boost the innate immune system by secreting interferon-γ in response to certain inflammatory cytokines[13], but whether these cells can substitute for naive T cells in antigen-specific responses is still unclear.

1. Hamilton, S.E., Wolkers, M.C., Schoenberger, S.P. & Jameson, S.C. *Nat. Immunol.* **7**, 475–481 (2006).
2. Kaech, S.M., Wherry, E.J. & Ahmed, R. *Nat. Rev. Immunol.* **2**, 251–262 (2002).
3. Cho, B.K., Rao, V.P., Ge, Q., Eisen, H.N. & Chen, J. *J. Exp. Med.* **192**, 549–556 (2000).
4. Goldrath, A.W., Bogatzki, L.Y. & Bevan, M.J. *J. Exp. Med.* **192**, 557–564 (2000).
5. Surh, C.D. & Sprent, J. *Semin. Immunol.* **17**, 183–191 (2005).
6. Bourgeois, C., Rocha, B. & Tanchot, C. *Science* **297**, 2060–2063 (2002).
7. Janssen, E.M. *et al. Nature* **421**, 852–856 (2003).
8. Shedlock, D.J. & Shen, H. *Science* **300**, 337–339 (2003).
9. Sun, J.C. & Bevan, M.J. *Science* **300**, 339–342 (2003).
10. Sun, J.C., Williams, M.A. & Bevan, M.J. *Nat. Immunol.* **5**, 927–933 (2004).
11. Janssen, E.M. *et al. Nature* **434**, 88–93 (2005).
12. Vos, Q., Jones, L.A. & Kruisbeek, A.M. *J. Immunol.* **149**, 1204–1210 (1992).
13. Berg, R.E., Crossley, E., Murray, S. & Forman, J. *J. Exp. Med.* **198**, 1583–1593 (2003).

Opn: key regulator of pDC interferon production

Wei Cao & Yong-Jun Liu

Plasmacytoid dendritic cells selectively express Toll-like receptors 7 and 9 and respond to virus infection by triggering massive interferon production, a pathway regulated by osteopontin expressed in plasmacytoid dendritic cells.

Plasmacytoid dendritic cells (pDCs), or natural type I interferon–producing cells, are specialized cells of the innate immune system that produce large amounts of type I interferon (IFN-α, IFN-β, IFN-ω and IFN-λ) after infection by a wide range of viruses[1,2]. These pDCs express Toll-like receptor 7 (TLR7) and TLR9, which trigger a cascade of signaling events leading to interferon gene transcription and secretion after engagement with microbial nucleic acids. Identifying molecules involved in this key TLR-interferon pathway has been an area of active research[3]. In this issue of *Nature Immunology*, Shinohara *et al.* provide genetic and biochemical evidence that osteopontin (Opn) is critical in regulating the TLR9-dependent pDC interferon response[4].

Opn (also called secreted phosphoprotein 1, early T lymphocyte activation 1, bone sialoprotein, urinary stone protein, nephropontin and uropontin) is encoded by a gene (*Spp1*) with high sequence homology among mammalian species[5]. Initially translated as a precursor of 314 amino acids with many negatively charged residues, most Opn undergoes complex post-translational modifications, including phosphorylation and glycosylation, before being secreted into various body fluids, where it can undergo further processing (human blood, for example, normally contains up to 20 ng/ml of Opn)[6]. Various cell types in the body express Opn, including osteoclasts and osteoblasts in bone tissue, cells in kidney, and interstitial fibroblasts and myocytes in the heart (especially after injury)[6], and thus diverse functions such as bone resorption, defense against renal stone formation and ventricular remodeling have been associated with Opn expression[5]. The importance of Opn for immune function has also been explored. For example, Opn has been characterized as functioning as a cytokine-like molecule[7], and *Spp1* transcription occurs constitutively in macrophages and is highly induced in T cells after signaling from the T cell receptor via T-bet, a 'master transcriptional factor' for T helper type 1 (T_H1) polarization[8]. Analysis of *Spp1*$^{-/-}$ mice has suggested that secreted, soluble Opn functions as an early component in the staging of effective T_H1 immune responses by differentially favoring expression of T_H1-driving interleukin 12 (IL-12) while simultaneously inhibiting expression of T_H2-skewing IL-10 in macrophages[9]. However, the mechanism underlying such a function for secreted Opn remains unclear.

In the study discussed here[4], Shinohara *et al.* report that Opn expression is induced in pDCs but not in conventional CD11c$^+$ DCs after treatment with CpG, a TLR9 agonist (**Fig. 1**). They find a possible link to T-bet expression after analysis of pDCs from wild-type and T-bet-deficient mice shows that T-bet is critical for Opn expression in both pDCs and T cells. Further experiments show that pDCs generated from either T-bet- or Opn-deficient mice produce much less IFN-α in response to CpG than do wild-type cells, whereas responses dependent on transcription factor NF-κB, such as IL-6 and tumor necrosis factor responses, are unaltered. Functional assays show that Opn is required for cross-presentation by pDCs, as Opn-deficient pDCs do not effectively cross-present antigen and, *in vivo*, Opn-deficient mice challenged with

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