with a neutralizing IL-17 mAb also resulted in increased lung CFU, and IL-22-deficient mice were resistant to infection. Lungs from infected IL-17- and IL-17R-deficient mice contained substantially reduced amounts of G-CSF, neutrophil recruitment and cellular infiltration as could be expected, but this may not have been the reason for the failure in protection. Instead the authors showed that IL-17 and IL-17R-deficient mice had strongly reduced expression of IFN-γ and IL-12p35 mRNA indicating that IL-17 was somehow required for the generation of a Th1 response (Figure 1). Their next series of experiments demonstrated that IL-17, either in the absence or presence of LVS, could directly induce the production of IL-12, IFN-γ, IL-6, KC and MIP-1α in bone marrow-derived DC (BMDC), sorted lung CD11c$^+$ cells, bone marrow-derived macrophages (BMDM), and freshly isolated alveolar macrophages. Coculture experiments of BMDC with naive OT-II transgenic T cells and OVA peptide showed that IL-17 could induce the polarization of these naive T cells into IFN-γ-producing Th1 cells. BMDC from IL-12p40-, IL-17R- and IFN-γ-deficient mice could not support this effect of IL-17 on T cell differentiation, indicating that it was specific and mediated through induction of IL-12 and IFN-γ.

These results demonstrate the IL-17 can regulate IL-12-Th1 cell immunity against an intracellular pathogen. This now turns the attention to the early events following infection as they set the stage for the ensuing immune response. The signals that give rise to IL-12 and IL-23 production by the DC will be important for determining a protective immune response. Are these cytokines produced by different types of DCs?

Timing is also an important factor because the induction of IL-12 and IFN-γ by IL-17 is a rapid and early event. The authors show that IL-17 is produced by antigen-specific T cells and by γδ T cells, which are part of the innate immune cells. Are the requirements for these cell types to produce IL-17 similar or different? And how do the IL-17-IFN-γ double producing T cells fit in? Without doubt, future investigations will focus on these and other aspects of the interaction between Th17 and Th1 cell pathways. These interactions will likely be complex because in the absence of DC, IL-17 has been shown to inhibit Th1 differentiation (O'Connor et al., 2009). However, this work now opens up insights in the IL-23-Th17 and IL-12-Th1 pathways and shows that there is not only counter-regulation but also interdependence.

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The Quest for CD8$^+$ Memory Stem Cells

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In this issue of *Immunity*, Turtle et al. (2009) describe the identification of a distinct CD8$^+$ memory T cell subset in humans, which could bring us closer to the identification of the enigmatic "memory stem cell."

CD8$^+$ T cells can confer protective immunity toward (intracellular) pathogens and some cancers. In order to maintain protection, long-living memory T cells are generated, which might persist throughout an individual's lifespan, probably without the need to re-encounter antigen (Williams and Bevan, 2007). The development of chronic disease after infection with common viruses such as cytomegalovirus (CMV) or Epstein-Barr virus (EBV) is prevented by the constitutive presence of virus-specific CD8$^+$ effector cells. Similarly, CD8$^+$ T cells are believed to contribute to virus control in long-term nonprogressing HIV-infected individuals. How exactly CD8$^+$ T cells are maintained beyond the contraction phase of an immune response is still only poorly understood. With the identification of functionally and phenotypically distinct subsets of memory T cells (Sallusto et al., 1999), however, a division of labor in between different cell types has

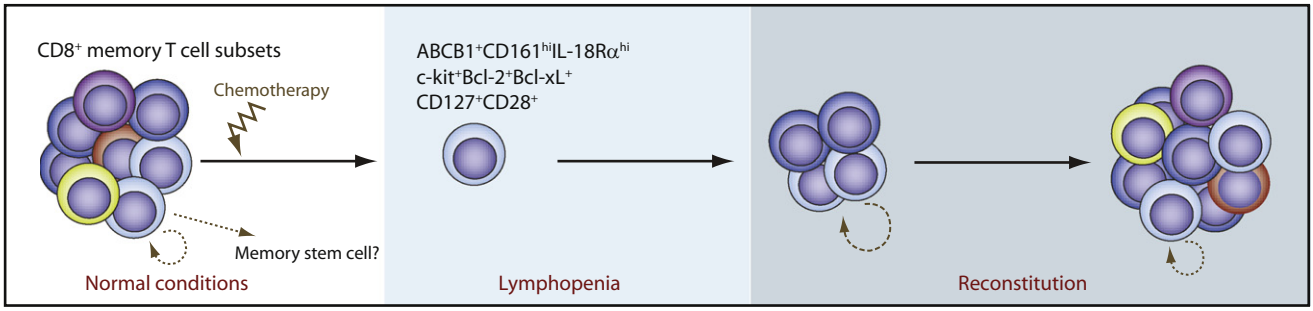

Figure 1. CD161^{hi}IL-18R^{hi} Memory T Cells Are Important for Reconstitution of the Memory T Cell Compartment
CD161^{hi}IL-18R^{hi} CD8$^+$ memory T cells are resistant to chemotherapy because of their expression of ATP-binding cassette (ABC)-superfamily multidrug efflux proteins (e.g., ABCB1). They are quiescent but have the capability of self renewal and proliferation and can differentiate into other cell subsets. These cells express stem cell-associated markers (c-kit), as well as antiapoptotic and survival proteins (Bcl-2 and Bcl-xL) or receptors (CD127). This particular subset of memory T cells might not only play a crucial role for immune reconstitution upon chemotherapy-induced lymphopenia, it could also be relevant for T cell memory maintenance under normal conditions.

become evident. The entire diversity of these subsets can be generated from a single naive precursor cell, and out of adoptively transferred single-cell-derived daughter cells, complex secondary immune responses can evolve (Stemberger et al., 2007). These findings—the enormous plasticity of CD8$^+$ T cell differentiation and the maintenance of this plasticity within specialized memory T cell populations—have initiated a debate about whether a "memory stem cell" exists. In the mouse, some phenotypical and molecular signatures (e.g., Wnt signaling) (Gattinoni et al., 2009) with similarities to naive T cells as well as hematopoietic stem cells (HSCs) have been found in distinct subsets of memory T cells, which in some cases might reside in specialized niches in the bone marrow (Mazo et al., 2005; Tokoyoda et al., 2009). However, a more conclusive definition of "memory stem cells" including (life-long) self-renewing capacity, which would have to be based on serial adoptive transfer experiments, still remains elusive.

The starting point of the studies by Turtle et al. (2009) in this issue of *Immunity* is the long-known clinical observation that repeated induction of profound lymphocytopenia in patients undergoing multiple cycles of cytotoxic chemotherapy only infrequently results in severe infections with viruses that can be controlled by memory T cells (such as CMV or EBV); this indicates that like HSCs, some CD8$^+$ memory T cells might be more resistant to chemotherapy and that they are fully capable of conferring or reconstituting protective immunity. The mechanisms by which HSCs are resistant to chemotherapy are related to both cell quiescence and the overexpression of ATP-binding cassette (ABC)-superfamily multidrug efflux proteins (e.g., ABCB1), which protect cells from toxic xenobiotics and endogenous metabolites. The authors initially searched for memory T cells able to efflux drugs or dyes that are substrates of the ABCB1 transporter, and they not only succeeded in identifying a small fraction of rapidly effluxing cells within the central memory (Tcm) and effector memory (Tem) compartments, they also uncovered two surface markers—namely CD161 and IL-18 receptor (IL-18R)—that allow selective labeling and further analysis of this unique human CD8$^+$ memory subset. CD161^{hi}IL-18R^{hi} cells preferentially survive exposure to chemotherapy in vitro and in vivo, are relatively quiescent but demonstrate proliferation and self-renewal in response to cytokines that maintain homeostasis, and differentiate in response to antigen stimulation into other effector and memory T cell subsets (Figure 1). In line with these characteristics, the unique memory subset expresses higher amounts of c-kit, Bcl-2, CD28, CD127, and Bcl-xL than other T cell subsets. Most importantly, within the CD161^{hi}IL-18R^{hi} subset, virus-specific CD8$^+$ T cells are readily detectable, and this cell type accumulates in the peripheral blood of patients shortly after chemotherapy, providing strong evidence that these cells are indeed crucially involved in providing protection and immune reconstitution.

Initial functional and phenotypic analyses of the CD161^{hi}IL-18R^{hi} CD8$^+$ memory T cell subset suggest that it is involved in clinically relevant situations such as immune reconstitution upon chemotherapy-induced lymphopenia. However, as the authors themselves state, a more conclusive demonstration of stem cell-like characteristics of this subset (such as serial adoptive transfers) will be required before a clear link to "memory stem cells" can be drawn; this will also require transmission of the findings to suitable animal models, where more conclusive experiments can be performed. Regardless of these limitations, with the currently available data it is tempting to speculate that the CD161^{hi}IL-18R^{hi} CD8$^+$ memory T cell subset is not only a crucial subset for immune reconstitution during temporary lymphopenia but also a central player in the maintenance of CD8$^+$ T cell memory in healthy individuals. With their stem cell-like character, the CD161^{hi}IL-18R^{hi} subset might be capable of constitutively feeding the pool of lifespan-limited and functionally diverse (effector) memory progenitor cells. In this case, maintenance of immunity by memory stem cells could be achieved by asymmetric cell division, as has been described for HSCs. CD161^{hi}IL-18R^{hi} CD8$^+$ memory T cells are found in low numbers circulating in the peripheral blood of healthy individuals, which might indicate that they do not need a specific niche for survival and self-renewal. Because CD161^{hi}IL-18R^{hi} CD8$^+$ memory T cells share homing receptors with Tcm and Tem cells, they will most likely migrate to similar sites while searching for their cognate antigen. Other groups have recently described that like plasma memory B cells, some CD8$^+$ and CD4$^+$ memory T cells are maintained in

specialized niches within the bone marrow (Mazo et al., 2005; Tokoyoda et al., 2009). Thus, the $CD161^{hi}IL\text{-}18R^{hi}$ $CD8^+$ memory T cell subset could also represent an early progenitor population released from true memory stem cells sitting in the bone marrow or other survival sites. In this case, cell subsets with the potential for immune reconstitution and long-term survival should preferentially be present within the more weakly differentiated Tcm cell compartment, which has also been demonstrated by Riddell and colleagues in primate studies (Berger et al., 2008). However, the even higher presence of the $CD161^{hi}IL\text{-}18R^{hi}$ $CD8^+$ memory T cell subset within the Tem cells does not fit that well into such a model. Therefore, it will be important to analyze whether bone-marrow-residing $CD8^+$ memory cells are distinct from the circulating $CD161^{hi}IL\text{-}18R^{hi}$ $CD8^+$ memory T cell subset and out of which compartment (Tcm or Tem cell) reconstituting cell populations really arise. Unraveling the exact relationship between different cell subsets as well as the identification of the underlying survival factors and micro-anatomical locations will become a major task for future research in the field. Furthermore, it will be interesting to investigate whether similar "stem cell-like" subsets can be found for other lymphocytes such as $CD4^+$ T cells, NK cells, or B cells.

The identification of a specific long-living $CD8^+$ T cell subpopulation that is chemotherapy resistant and can reconstitute pre-existing immunity has important clinical implications, especially for vaccination and adoptive immunotherapy. The detection of $CD161^{hi}IL\text{-}18R^{hi}$ $CD8^+$ memory T cells after vaccination might allow monitoring of a sustained immune response and could help to improve ineffective vaccination strategies. For adoptive immunotherapy with ex vivo-isolated (Knabel et al., 2002) or in vitro-expanded cells, the presence of this unique cell population in the transplant should be advantageous for sustained therapeutic effects; if this holds true, the presence of $CD161^{hi}IL\text{-}18R^{hi}$ $CD8^+$ memory T cells could be used to identify optimal donors and might become an important quality control measure for clinical T cell products. Additional evidence for this assumption has just been provided by the observation that upon bone marrow transplantations, the phenotype of donor-derived CMV-specific $CD8^+$ T cells, especially the presence of a subset of $CD27^+CD57^-$ memory T cells (a surface expression pattern also shared by $CD161^{hi}IL\text{-}18R^{hi}$ cells), positively correlates with a lower risk to develop post-transplant CMV-associated complications (Scheinberg et al., 2009). Obviously, this is going to be an exciting and clinically important area for future research.

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