6. Kakonen, S.M. *et al. J. Biol. Chem.* **277**, 24571–24578 (2002).
7. Erlebacher, A., Filvaroff, E.H., Ye, J.Q. & Derynck, R. *Mol. Biol. Cell* **9**, 1903–1918 (1998).
8. Filvaroff, E. *et al. Development* **126**, 4267–4279 (1999).
9. Lucas, P.A. *Bone* **10**, 459–463 (1989).
10. Gupta, S. & Cheikh, I.E. *Endocr. Pract.* **11**, 399–407 (2005).
11. Kurihara, N. *et al. J. Bone Miner. Res.* **21** Suppl 2, 55–57 (2006).
12. Mohammad, K.S. *et al. PLoS One* **4**, e5275 (2009).

Tumor immunotherapy: making an immortal army

Brent H Koehn & Stephen P Schoenberger

Manipulation of cell renewal pathways creates T memory stem cells that can generate a sustained and targeted immune response. These findings have broad implications for vaccine development and immunotherapy.

The Greek historian Herodotus tells of an elite corps of Persian warriors who fought the vastly outnumbered Spartans at the battle of Thermopylae in 480 BCE[1]. Herodotus called these fabled Persians the "immortals," because as each man fell to disease or injury, he was immediately replaced by another well-trained soldier, maintaining the numbers of the force at a constant strength[1].

In this issue of *Nature Medicine*, echoes of this epic story appear in a new immunotherapy tactic. Gattinoni *et al.*[2] have found a way to generate optimal CD8$^+$ T cell responses[2]—through activation of the Wnt–β-catenin pathway. Activation of this pathway can convert CD8$^+$ T cells into a self-renewing 'army' of stem cells that generates specialized effector T cells capable of eradicating tumors many thousands of times larger on a per-cell basis[2]. If reproducible in the clinical setting, this strategy could markedly improve the efficacy of both vaccines and adoptive immunotherapies through the continuous generation of antigen-specific CD8$^+$ T cells from a self-renewing reservoir.

CD8$^+$ T cells are the 'killer' lymphocyte subset, charged with executing virtually any cell in the body that may become infected with an intracellular pathogen. They do this by inducing apoptosis through a variety of mechanisms and have been shown to be remarkably effective against tumors that express mutated self-proteins or tissue-specific differentiation antigens[3]. As such, they represent a powerful weapon within the immunotherapist's arsenal.

Antigen-specific CD8$^+$ T cells can be isolated from the blood of patients with cancer and expanded *in vitro* to large numbers that can be transferred back to the patient and attack the tumor. Alternatively, these cells can be readily induced within an individual through vaccination.

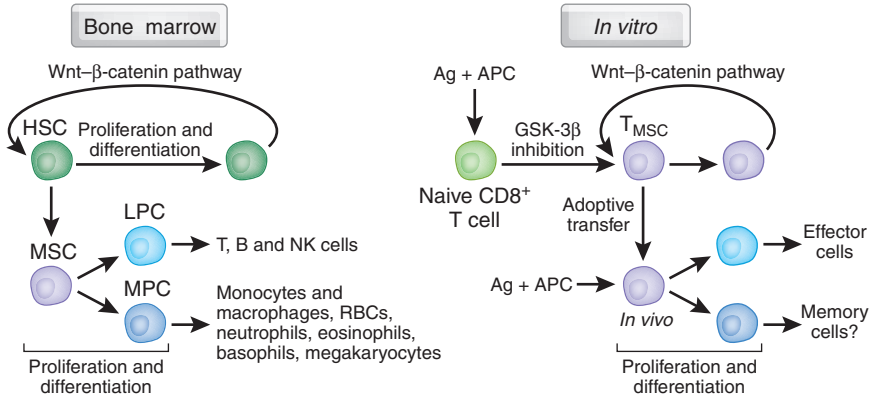

Figure 1 Same pathway, different outcomes? During hematopoiesis in the bone marrow, the Wnt–β-catenin pathway limits the proliferation and differentiation of hematopoietic stem cells (HSCs) so that division can regenerate HSCs in addition to the multipotent stem cells (MSCs) that give rise to lymphoid progenitor cells (LPCs) and myeloid progenitor stem cells (MPCs). Gattinoni *et al.*[2] induced this pathway in mature CD8$^+$ T cells through pharmacological inhibition of GSK-3β during priming, resulting in the generation of memory cells that possess stem cell–like qualities of self-renewal and multipotency. NK, natural killer; RBCs, red blood cells; Ag, antigen; APC, antigen-presenting cell.

The rapid disappearance of CD8$^+$ T cells after vaccination or adoptive transfer, however, limits their potential efficacy. After primary activation by antigen, naive T cell precursors undergo clonal expansion to generate a range of functionally distinct subsets of T cells that differ in their longevity, location and cytotoxic potential. Most of the CD8$^+$ T cells that arise from vaccination and during *in vitro* expansion are short-lived effector cells that are terminally differentiated. Such cells can effectively kill the first wave of targets they encounter, but, in the absence of memory cells able to persist and generate new effectors, the immune response will be temporary at best.

The capacity for self-renewal and continued differentiation is found within two other subsets of T cells: the effector memory cells (T_{EM} cells) found mostly in peripheral tissues and the central memory cells (T_{CM} cells) that reside in lymphoid organs such as spleen and lymph nodes. How these memory subsets are induced and maintained, however, is unclear[4].

The Wnt pathway involves a number of evolutionarily conserved proteins that regulate many cellular events, ranging from embryogenesis to differentiation[5]. Binding of Wnt proteins to cell surface receptors leads to a change in the amount of β-catenin (an intracellular signaling molecule) that reaches the nucleus, where it interacts with members of the TCF/LEF family of transcription factors to promote new gene expression[6]. In hematopoietic stem cells, Wnt controls self-renewal by limiting proliferation and differentiation so that division can regenerate both multipotent daughter cells and additional pluripotent stem cells[7].

In their search for a sustained immune response against particular tumor antigens, Gattinoni *et al.*[2] investigated whether mature T cells could become memory cells after activation of the Wnt–β-catenin pathway. The researchers treated a line of T cells that specifically recognize a melanoma antigen with several small molecules that activate the

Brent H. Koehn and Stephen P. Schoenberger are in the Laboratory of Cellular Immunology, La Jolla Institute for Allergy and Immunology, La Jolla, California, USA.
e-mail: sps@liai.org

Wnt–β-catenin pathway, such as an inhibitor of glycogen synthase kinase-3β (Gsk-3β), which represses Wnt signaling[2]. Activation of the Wnt pathway inhibited both the proliferation of the treated cells and their ability to kill target cells. What's more, the percentage of cells expressing markers of T_{CM} cells and stem cells increased.

After adoptive transfer and antigenic stimulation, the treated cells rapidly differentiated into effector $CD8^+$ T cells, producing cytokines and undergoing substantial proliferation. Moreover, small numbers of transferred T cells could induce the destruction of tumors derived from B16 melanoma cells better than inoculation with either T_{EM} or T_{CM} cells. Importantly, some cells continued to express T_{CM} markers after multiple rounds of division, indicating they had retained their 'stemness' and thereby earning the designation of T memory stem cells (T_{SCM} cells)[8].

If the investigators included Gsk-3β inhibitors during vaccination of mice *in vivo*, there was an increase in the percentage of $CD8^+$ T cells with markers of the T_{CM} cells[2], although this cannot be unambiguously ascribed to a direct effect on $CD8^+$ T cells themselves rather than another cell type. If true, however, this observation suggests that activating the Wnt–β-catenin pathway could induce the formation of T memory stem cells during the initial priming step of immunization, leading to potent and sustained cytotoxic T cell responses at the next exposure of antigen.

Taken together, the results of Gattinoni *et al.*[2] outline a new approach to optimize $CD8^+$ T cell responses, and these findings have the potential for clinical application for vaccines and adoptive immunotherapy. Generating T_{SCM} cells means that fewer cells may be required for adoptive immunotherapy, making a complex technique less cumbersome and potentially more widely used. Similarly, vaccines supplemented with Gsk-3β inhibitors could produce a more durable immunity against infectious agents—one that isn't susceptible to terminal differentiation and the 'exhaustion' of T cells that weakens immunity over time.

Much work remains before the physiology and molecular regulation of this phenomenon is fully understood. Only a subset of the total responders become T_{SCM} cells, leading Gattinoni *et al.*[2] to speculate that asymmetric inheritance of signaling molecules during cell division may be involved in the acquisition of this cell fate. The direct visualization of these events may reveal the answer to this question. Furthermore, although the authors assume that inhibiting Gsk-3β induces activation of the Wnt–β-catenin pathway[2], other signaling and downstream events could play a part in the generation of the T memory stem cells. A genetic approach might confirm the molecular pathways that are active in this system.

That central memory T cells are generated after Gsk-3β inhibition *in vivo* raises the question of whether this pathway normally operates during memory formation and whether it occurs in a specific environment or niche. Wnt signaling is active in the bone marrow to restrain the differentiation of hematopoietic stem cells, but it is unknown whether memory T cells also use this pathway for their generation. Finally, these results lend themselves to the ongoing debate on the lineage relationship between effectors and memory cells by showing that acquisition of cytotoxic function need not occur for cells to develop into T_{SCM} cells, at least not under conditions of Gsk-3β inhibition. Whether these findings are a faithful replication of a physiological pathway or a beneficial outcome of strategic pharmacology will be decided by the studies that will undoubtedly be initiated by this report.

1. Herodotus. *Histories* (de Selincourt, A., trans.) edn. 11 (Penguin, London, 1972).
2. Gattinoni, L. *et al. Nat. Med.* **15**, 808–813 (2009).
3. Berke, G. *Annu. Rev. Immunol.* **12**, 735–773 (1994).
4. Surh, C.D. & Sprent, J. *Immunity* **29**, 848–862 (2008).
5. Croce, J.C. & McClay, D.R. *Methods Mol. Biol.* **469**, 3–18 (2008).
6. Mosimann, C., Hausmann, G. & Basler, K. *Nat. Rev. Mol. Cell Biol.* **10**, 276–286 (2009).
7. Malhotra, S. & Kincade, P.W. *Cell Stem Cell* **4**, 27–36 (2009).
8. Stemberger, C. *et al. Semin. Immunol.* **21**, 62–68 (2009).

Targeting lymphotoxin depletes pathogenic T cells

Anna M Hansen & Rachel R Caspi

A monoclonal antibody directed against lymphotoxin-α (LT-α) expressed by pathogenic T cells can prompt the clearance of these cells from the body (pages 766–773). The findings bring us one step closer to targeting only the cell populations that cause harm in autoimmune diseases while leaving beneficial arms of the immune system largely intact.

Over the last ten years, the treatment options for individuals with autoimmune disorders have improved dramatically. A range of protein-based therapeutic agents have entered the market that specifically target the signaling pathways that cause conditions such as rheumatoid arthritis and multiple sclerosis. These therapies include agents that directly neutralize inflammatory cytokines, interfere with T cell activation or lead to the depletion of specific cell types known to be involved in these diseases.

Although the benefits of these therapies are indisputable, they can come at a high price to the patient. In addition to targeting the pathological inflammation, these immunotherapeutic agents can also interfere with normal host defense against infection and malignancy[1]. Therefore, enhancing target specificity has been one of the primary goals of the field.

In this issue of *Nature Medicine*, Chiang *et al.*[2] report the identification of an antibody that specifically targets activated T cell subsets involved in autoimmunity and propose that this new approach may lead to a substantial improvement over current treatments.

We rely on our immune system to fight infection and to clear cells that have died or that have become malignant, but the same mechanisms that protect us can also bring harm. When the complex balance of regulation and responsiveness breaks down, lymphocytes begin to attack self antigens, leading to autoimmune destruction of the tissue. Consequently, therapeutic agents that target autoimmune cells tend to be detrimental to normal antimicrobial defense, because the same functions underlie both the normal and the pathologic immune processes. For example, tumor necrosis factor-α (TNF-α), which is a therapeutic target in rheumatoid arthritis and several other autoinflammatory diseases, is expressed by almost all cells of the immune system. TNF-α is essential in defending

Anna M. Hansen and Rachel R. Caspi are in the Laboratory of Immunology, National Eye Institute, National Institutes of Health, Bethesda, Maryland, USA.
e-mail: rcaspi@helix.nih.gov