The Stem of the T

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The ability of the immune system to recognize pathogens to which it was previously exposed depends on the formation of immunologic memory. Upon initial antigen recognition and activation, T cells expand and differentiate to effector T cells that mediate cytotoxic effects to clear the infection. Most effector T cells die, but subsets of memory T cells termed central and effector memory cells are formed during the primary response and patrol lymph nodes, blood and tissue sites for the life of the individual. These memory T cells have been proposed to function as stem cells by maintaining the ability to self-renew and differentiate into new effector T-cells in response to antigen re-encounter, but whether this function is provided by all memory subsets has been controversial. The ultimate proof of stemness requires the isolation, adoptive transfer, and fate mapping of individual cells to test for both self-renewal and multipotency. Using three generations of single-cell adoptive transfer and infection-driven expansion, a new study published in *Immunity* identifies the putative stem cell that provides immune memory as the central memory T cell.

The experimental capability for this study was developed through a long-term collaboration, focused on clinical cell processing and purification, between researchers based in Munich, Germany and Seattle. This team included Dr. Stanley Riddell from the Clinical Research Division and senior fellow Hans Fischer and colleagues at the Institute for Advanced Study of the Technical University of Munich. The researchers assessed the stemness of isolated subsets of CD8 T cells. The separation of the subtypes of memory T cells extended from previous research in the Riddell laboratory that suggested the existence of memory T cells with stem-cell-like characteristics that protect chemotherapy patients from infection when the rest of their immune system is ablated (Turtle *et al*., 2009). Memory T cells were isolated from the blood based on expression of specific cell surface markers, including CD62L. Central memory T cells (Tcm) express CD62L and are less prevalent in the blood while effector memory T cells (Tem) are CD62L-negative.

In this study, single naïve T cells were first transferred into mice and then exposed to the bacteria *Listeria monocytogenes* to instigate formation of memory cells. At least two months after the initial infection subsided, the researchers isolated and transferred single Tcm cells that were derived from the progeny of the single naïve T cell and specific for *Listeria* into either immunocompetent or immunocompromised mice. The individual Tcm cells generated progeny of equal number and diversity upon secondary transfer after antigen re-exposure as the naïve T cell did upon first transfer. Furthermore, transfer of just ten secondary Tcm cells was sufficient to protect immunocompromised mice from a lethal challenge of *Listeria monocytogenes*.

Only 20% of the mice developed T cell progeny after single naïve T cell or Tcm cell transfer, similar to the recovery rate of single hematopoietic stem cells, demonstrating the technological feat of these experiments. Importantly, the researchers found that not even 100 Tem could be propagated across serial adoptive transfers. This result supports a model of progressive differentiation in which the Tem and effector T cells that differentiate from Tcm lose self-renewal capabilities, while Tcm are maintained by self-renewal.

According to Dr. Riddell this paper "provides the first proof that central memory T cells are in essence 'stem cells' for an epitope specific immune response providing a mechanism for the longevity/durability of T cell memory." These studies also have implications for immunotherapy and current research by the Riddell Laboratory is harnessing the power of the immune system by redirecting specific subsets of T cells to recognize and kill tumor cells. T cells are harvested from patients and genetically engineered to target tumor cells in the laboratory, and then transferred back into patients. Specifically isolating and reprogramming CD62L-positive Tcm cells may improve the persistence and efficacy of adoptive T cell therapies. The study by Graef *et al*. further shows that smaller numbers of these cells could be used to reduce the cost of the treatments and expand the application of adoptive T cell therapy. Current clinical trials are underway to evaluate if use of these memory cells translates into increased tumor responses.

Graef P, Buchholz VR, Stemberger C, Flossdorf M, Henkel L, Schiemann M, Drexler I, Höfer T, Riddell SR, Busch DH. 2014. Serial transfer of single-cell-derived immunocompetence reveals stemness of CD8(+) central memory T cells. *Immunity* 41:116-126.

See Also: Gattinoni L. 2014. Memory T cells officially join the stem cell club. *Immunity* 41:7-9.

Turtle CJ1, Swanson HM, Fujii N, Estey EH, Riddell SR. 2009. A distinct subset of self-renewing human memory CD8+ T cells survives cytotoxic chemotherapy. *Immunity* 31:834-844.

Turtle CJ, Hudecek M, Jensen MC, Riddell SR. 2012. Engineered T cells for anti-cancer therapy. *Current Opinion Immunology* 24:633-639.

"Souped-up CARs drive T-cell killing of ROR1-positive tumors" June 17, 2013 in *Science Spotlight*

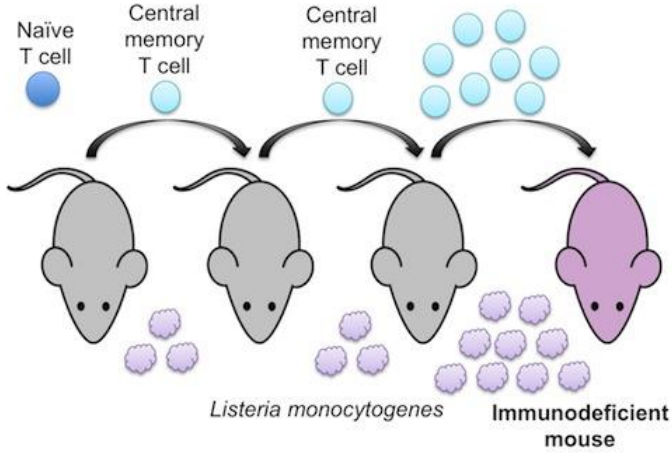

Image adapted from Gattinoni, 2014

Serial adoptive transfer of single memory T cells positive for cell surface marker CD62L, followed by infection-driven expansions, demonstrates stemness of these memory T cell subsets. These central memory T cells are capable of protecting immunodeficient hosts from lethal bacterial challenge.