Stem Cell Gene Therapy Blocks HIV Infection and Boosts Antiviral Immunity

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A major obstacle to control and cure HIV is the persistence of latent viral reservoirs within the bodies of infected people. Highly active antiretroviral therapy (HAART) reduces the spread of HIV by inhibiting viral replication, but does not eliminate these viral reservoirs. Unfortunately, some HIV-infected individuals cannot tolerate long-term HAART due to severe side effects, or develop multidrug resistant virus. In one remarkable instance, an HIV-positive patient was "cured" of infection while being treated for acute myeloid leukemia. This patient received a stem cell transplant from a donor who had a rare genetic mutation that makes cells resistant to HIV infection. The mutation in the gene CCR5 prevented the expression of the CCR5 co-receptor necessary for HIV binding and entry into cells. Unfortunately, this approach is limited in its applicability since finding a matched donor for transplant is extremely rare and allogeneic stem cell transplantation has significant associated morbidities. However, the elimination of HIV in one patient suggests that genetically engineering a patient's own immune cells to be HIV-resistant could prove to be a viable therapeutic strategy. Lead author Dr. Patrick Younan and colleagues in the laboratory of Dr. Hans-Peter Kiem (Clinical Research Division) demonstrate for the first time the possible benefits of this strategy to treat HIV-positive patients in a clinically relevant nonhuman primate AIDS model.

Clinical trials are under way using zinc finger nucleases that specifically target and inactivate the CCR5 gene in peripheral T-cells of HIV-infected patients. However, other HIV strains use a different co-receptor, CXCR4, and would not be inhibited by this strategy. An alternative strategy for making cells resistant to HIV infection is genetically-engineering cells to express a fusion inhibitor, mC46. By binding the viral protein gp41, mC46 disrupts the HIV molecular machinery needed for the fusion of the viral membrane to the host cell membrane. Initial clinical trials demonstrated that modifying a patient's T-cells to express mC46 is well tolerated in HIV-positive patients (Van Lunzen K *et al*., 2007). An improved strategy would be to express mC46 on hematopoietic stem cells (HSCs), which would provide protection to both lymphoid and myeloid cells susceptible to HIV infection and provide a source of long-lived modified progenitor cells.

Mouse models of HIV infection facilitate rapid functional testing of the gene-modified cells, but nonhuman primate models are necessary to determine the conditions necessary to achieve sufficient stem cell engraftment and long-term protection from HIV in humans. Younan *et al*. transplanted four pigtail macaques with genetically-modified HSCs with identical pre- and post-transplant conditioning regimens. Two animals received control HSCs and two animals received mC46-expressing HSCs. Both control and mC46-expressing HSC were engineered to express a chemotherapy-resistance gene and green-fluorescent protein (GFP) with a lentiviral vector to enrich for the modified cells *in vitro* by GFP and *in vivo* by chemotherapy treatment. This treatment allowed for sufficient numbers of modified cells to recover in the animal for two weeks before infection: one animal recovered with 20% modified CD4+ T-cells (mC46.1) and the other animal 55% (mC46.2).

All animals were infected with a highly pathogenic chimera HIV/SHIV strain that uses both CCR5 and CXCR4 as co-receptors. During the acute phase of the infection, there was an initial drop in mC46-modified T-cell numbers, most likely due to bystander mediated-apoptosis and not direct infection. Three weeks after infection, both mC46.1 and mC46.2 macaques increased mC46-modified CD4+ T-cells to greater than 90% of the total T-cell numbers, while no selective advantage was observed in the control animals. At five months after infection the researchers also found recovery of CD4+ T-cells that were not modified to express mC36.1 close to normal levels in the monkeys that received mC46.1-modified HSCs, a result not found in mouse AIDS models. A more moderate increase in unmodified CD4+ T-cells was observed in mC46.2 that could be due to the higher percentage of modified T-cells recovered before infections.

Strikingly, an enhanced immune response was observed in the mC46 transplanted macaques, with maintenance of SHIV-specific CD4+ T-cells and cytotoxic CD8+ T-cells, and antibody responses against the infected SHIV strain. Six months after SHIV-infection, serum samples showed neutralization of viral infection with more than four-fold activity in mC46 macaques versus controls. The researchers propose the preservation of SHIV antigen-specific CD4+ T cells can contribute to boosting the development of virus-specific memory CD8+ T-cells and neutralizing antibodies. Studies of human patients who are long-term non-progressors and elite controllers of HIV infection suggest that maintenance of HIV-specific CD4+ T-cells enhances the ability of the immune system to suppress viral replication. As such, the mC46 macaques did not show disease progression to AIDS in the absence of antiretroviral treatment. Plasma viremia was 320-fold lower in the mC46.1 at day 168, and 1,477-fold lower in mC46.2 at day 20 compared to their respective controls (p=0.02).

According to Drs. Kiem and Younan, "these results suggest that genetically modifying hematopoietic stem cells not only results in the protection of gene-modified immune cell populations, but also provides these infection-resistant cells the opportunity to elicit an enhanced immune response against the virus." Since this study introduced modified stem cells before viral infection, it will be necessary to examine the protective effect in animals infected with SHIV/HIV prior to transplant. Realistically, human treatment of this nature would be initiated after initial infection. Notably, mC46 is structurally similar to the FDA-approved fusion inhibitor enfuvirtide. HIV can mutate and become resistant to the effects of enfuvirtide in the absence of additional antiretroviral therapies in humans; therefore, it is possible that mC46-modified immune cells may also elicit HIV strains resistant to mC46 binding. Nevertheless, Drs. Kiem and Younan provocatively ask, "Is it possible that by rendering immune cells resistant to infection and enhancing the immune response to HIV that we may be able to develop strategies to also eradicate HIV?"

Younan PM, Polacino P, Kowalski JP, Peterson CW, Maurice NJ, Williams NP, Ho O, Trobridge GD, Von Laer D, Prlic M, Beard BC, Derosa S, Hu SL, Kiem HP. 2013. Positive selection of mC46-expressing CD4+ T cells and maintenance of virus specific immunity in a primate AIDS model. *Blood*. Epub ahead of print; doi: 10.1182/blood-2013-01-482224.

Also see: Kiem HP, Jerome KR, Deeks SG, McCune JM. 2012. Hematopoietic-stem-cell-based gene therapy for HIV disease. Cell*Stem Cell* 10:137-47.

van Lunzen J, Glaunsinger T, Stahmer I, von Baehr V, Baum C, Schilz A, Kuehlcke K, Naundorf S, Martinius H, Hermann F, Giroglou T, Newrzela S, Müller I, Brauer F, Brandenburg G, Alexandrov A, von Laer D. 2007. Transfer of autologous gene-modified T cells in HIV-infected patients with advanced immunodeficiency and drug-resistant virus. *Molecular Therapy* 15:1024-33.

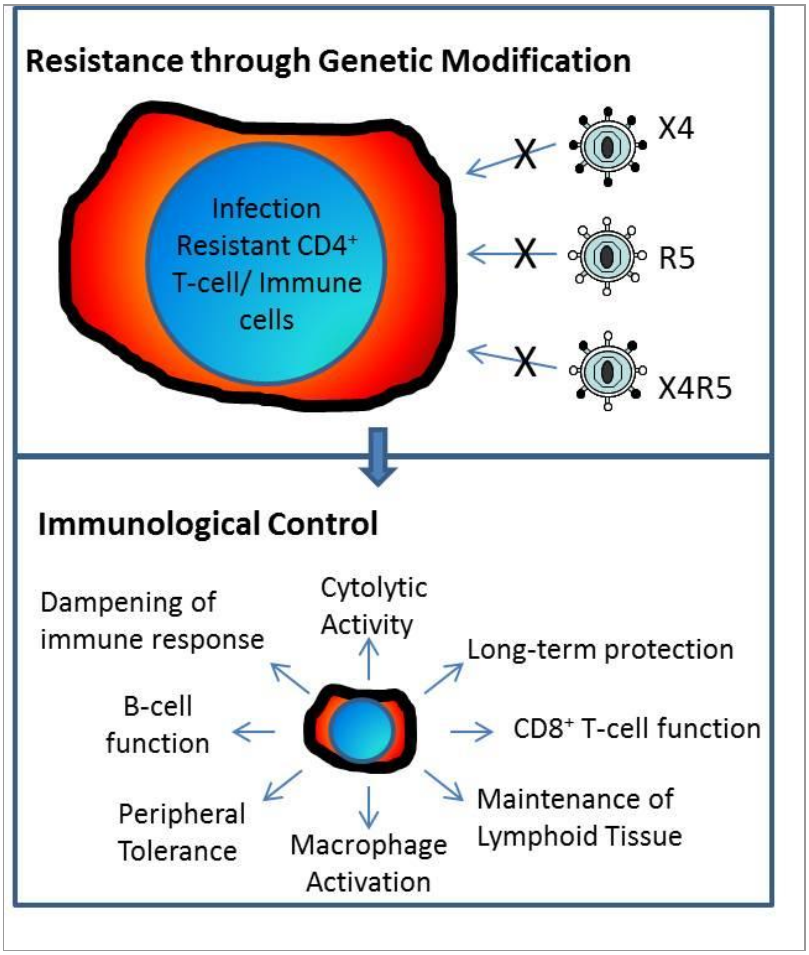

Image provided by Drs. Patrick Younan and Hans-Peter Kiem

Genetic modification of immune cells to express the fusion inhibitor mC46 blocks infection of all HIV strains with tropism for CCR5 (R5), CXCR4 (X4) or both co-receptors (X4/R5). Sparing a subset of CD4+ T-cells from infection boosts the immune response against HIV through various mechanisms, including enhanced cytotoxic T-cell and antibody responses.