Establishment of macaque CD4+ T cells and CD34+ hematopoietic stem cells resistant to SIV infection using Zinc Finger Nucleases technology

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Background: CCR5 is the major HIV co-receptor, and individuals homozygous for a 32-bp deletion in Ccr5 gene are resistant to infection by CCR5-tropic HIV-1. Therefore, the CCR5 co-receptor provides a unique opportunity to exploit gene knockout technologies for anti-HIV therapy. The Berlin patient highlights the potential therapeutic benefit of CCR5 disruption in treatment and possible eradication of HIV infection.

Methods: Various gene therapy approaches to block CCR5 expression are currently being evaluated. The targeted cell populations include both mature peripheral T cells and Hematopoietic Stem Cells (HSC). The loss of CCR5 in HSC appears to have no adverse effects on hematopoiesis. We are pursuing the use of engineered Zinc Finger Nucleases (ZFNs) to permanently disrupt the CCR5 open-reading frame. In our system, the ZFN set chosen generates a DNA double-strand break in the region encoding for the second extracellular loop of CCR5, thus mimicking a CCR5delta32 mutation. CCR5-targeted ZFNs are evaluated *in vitro* in our laboratory, targeting mature CD4+ T cells and hematopoietic stem cells isolated from naïve-uninfected macaque blood, bone marrow and umbilical cord samples.

Results: We engineered SIV-resistant macaque CD4+ T cells using CCR5-ZFNs. After nucleofection of mRNAs encoding for ZFNs into CD4+ cells isolated from macaques, we show that these cells were resistant to in vitro SIVmac239, SIVmac251, and SIVagm infections as shown by the absence of p27 expression. We then focused on the modification of HSC isolated from macaque femoral bone marrow and umbilical cords. We established conditions required to purify and grow macaque CD34+ HSCs in vitro to maximize the efficiency of CCR5 gene disruption while minimizing any adverse effects on cell viability or hematopoietic potential. We successfully engineered CCR5-modified macaque hematopoietic stem cells that were resistant to SIVmac239 infection after in vitro differentiation and expansion on thymocytes.

Conclusion: We demonstrated the feasibility of using ZFN technology to establish CD4+ T cells and hematopoietic stem cells resistant to SIV infection in macaque. The generation of a nonhuman primate model using this modern molecular-based strategy might significantly help in the design of new therapies to prevent viral infection and eradicate HIV infection in human.