Counterpoint: Cord Blood Stem Cell Therapy for Acquired Immune Deficiency Syndrome

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GONZALEZ ET AL. PROPOSE the *in vivo* use of cord blood stem cells isolated from individuals who are homozygous for a 32-base pair deletion ($\Delta 32$) in the CCR5 gene as a potential therapy for AIDS. The overall goal of this transplantation strategy is to generate CD4⁺ T lymphocytes that resist HIV-1 infection and restore the immune system in HIV patients.

While the idea of generating resistance to HIV is very intriguing, there are a number of scientific findings to consider. The defective CCR5 gene containing the deletion encodes a prematurely terminated protein that is not detected at the cell surface [1–5]. We have demonstrated that this truncated mutant protein is indeed expressed in CCR5^{–/–} subjects [6,7]. Recent studies suggest that expression and stability of the CCR5 $\Delta 32$ protein are critical for developing the observed resistance [6]. It has been proposed that resistance to HIV-1 in CCR5 $\Delta 32$ homozygotes may result from both genetic loss of CCR5 on the cell surface as well as active downregulation of CXCR4 expression by the mutant CCR5 $\Delta 32$ protein [7]. Therefore, it will be critical to test whether the donor cord blood sample expresses this HIV suppressive factor, the CCR5 $\Delta 32$ protein. There is also concern about the availability of cord blood samples that are homozygous for the deleted CCR5 allele. While one copy of the mutant allele occurs at high frequency in certain human populations [8] the reported frequency of the homozygous genotype is much lower. CCR5 $\Delta 32$ generally is found in populations of European descent, with allelic frequencies ranging from 0 to 0.29 (~3% of Caucasians) [9]. Additionally, it was previously determined that the mutant CCR5 $\Delta 32$ allele is present at a frequency of ~0.10 (1%) in the Caucasian population of the United States [2].

The finding that HIV-1 infection has been reported in some individuals who are homozygous for the mutant CCR5 allele lowers the enthusiasm for the cord blood stem cell transplantation approach. HIV⁺ individuals have been identified in some hemophiliac CCR5^{–/–} patients [10] and several CCR5^{–/–} homosexuals [11–17] indicating that the protective effect of the CCR5 $\Delta 32$ mutation is not absolute. However, it could be argued that these findings should not lower our enthusiasm to transplant cord blood stem cells carrying the CCR5 $\Delta 32$ homozygous genotype. First, these HIV⁺ CCR5^{–/–} subjects are very rare and do not represent

a significant number compared to the majority of CCR5^{–/–} subjects who are HIV[–]. Second, the mechanism of the loss of the protective effect in the HIV⁺CCR5^{–/–} subjects is still unclear and requires further investigation. Our data demonstrated that some of these subjects do not express the CCR5 $\Delta 32$ protein [6,7].

Despite the potential adverse outcome of the cord blood stem cell transplantation, I believe that the proposed idea by Gonzalez et al. is worth testing in a mouse model system reconstituted with CD34⁺ stem cells isolated from CCR5^{–/–} cord blood cells. Previous studies described certain strains of mice lacking genes critical for the development of lymphocytes have been given human stem cells at birth, which differentiate into all of the known human lymphocyte subpopulations [18]. The “humanized” mice appear to have a normal immune system with normal systemic distribution of the human lymphocytes. Using human cord blood stem cells that are homozygous for the CCR5 $\Delta 32$ will probably determine whether it is possible to generate a human immune system that is resistant to HIV infection. I think developing this system will be necessary to understand the mechanism of resistance *in vivo*. It will be critical to determine whether the transplanted cord blood stem cells will differentiate and give rise to lymphoid cells that are resistant to X4 and R5 HIV-1 infection.

Previous data in the literature provided optimism that resistance to HIV can be modeled in a humanized mouse model by reconstituting the animals with peripheral blood lymphocytes (PBLs) derived from CCR5 $\Delta 32$ homozygous and heterozygous subjects [19,20]. These studies reported resistance of the transplanted cells to R5 but not to X4 strains. It was found that the extent of T-cell dysfunctions induced by a X4 strain of HIV-1 in SCID mice reconstituted with human PBLs was related to the *in vivo* state of activation of the human lymphocytes [19]. It will be interesting to test whether humanized mice reconstituted with CCR5^{–/–} cord blood stem cells would be resistant to both X4 and R5 strains of HIV-1.

Interestingly, the first successful allogeneic stem cell transplantation in an HIV-positive patient has recently been reported at the 15th Conference on Retroviruses and Opportunistic Infections in Boston, Massachusetts [21]. The

donor stem cells originated from a healthy HIV-negative CCR5^{-/-} individual. The patient managed transplantation without any remarkable irregularities and developed a functional reconstitution of his T-cell immunity. The transplanted patient showed no detectable HIV virus during a 22-month follow-up period. Although this case provided the proof of principle experiment, it remains a single case and the long-term effects of such treatment are still unknown.

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