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Lentiviral vectors

Lentiviral vectors for the treatment of Wiskott–Aldrich syndrome

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Inherited disorders affecting the blood-forming tissues could be treated if a gene could be transferred into stem cells capable of regenerating bone marrow and lymphoid organs and its expression regulated in differentiated cell populations. Molecular Therapy reports on new progress in the development of a vector for transferring a therapeutic gene for one such disorder, Wiskott–Aldrich syndrome (WAS), an X-linked disorder in which boys are variably affected with a triad of symptoms. Defects in the immune system render boys with WAS susceptible to infections, a low platelet count creates risk for serious bleeding, and recurrent skin rashes are problematic. Older boys may develop lymphoid cancer or, paradoxically, the immune system may turn on cells of their own bodies, resulting in an autoimmune disorder. Life expectancy is only 20 years. Bone marrow transplantation is curative, but many boys lack a matched donor and older boys are at high risk for transplant complications.

Dupré *et al*[1] have recently reported important progress in the development of vectors suitable for transferring the gene for the protein that is deficient in WAS. This protein (WASP) is expressed predominantly in cells of the blood system.[2] It transmits and integrates signals arising at the cell membrane that result in shape changes or cell movement through reorganization of the actin cytoskeleton. WASP is directly involved in forming the immunological synapse on T-lymphocytes that interact with specific antigens displayed on presenting cells.[3] Earlier studies showed that oncoretroviral vectors based on a mouse leukemia virus are able to correct functional defects in T-lymphocytes of patients with WAS.[3–5] Such vectors also improve, but do not fully correct, defects in the immune system in genetically modified mouse strains having WAS.[6,7] A defect in T-cell-mediated immunity to influenza was functionally corrected[7] but WASP$^-$ mice that had received gene-corrected stem cells remained susceptible to pneumococcal and mycobacterial challenge (T Strom, personal communication).

In an effort to improve gene transfer efficiency, Dupré *et al*[1] have exploited the properties of lentiviral vectors derived from the human immunodeficiency virus (HIV). The preintegration complex (PIC) of such vectors is relatively stable, prolonging the period during which vector integration within the stem cells can occur. Furthermore, the PIC is able to pass through the nuclear membrane without cellular mitosis.[8] These features contrast with those of mouse oncoretroviral vectors that have been relatively inefficient at transducing quiescent human stem cells or lymphocytes. Recent studies in a non-human primate model showed, as predicted, improved efficiency of gene transfer into repopulating, blood-forming stem cells with a lentiviral vector.[9]

In an attempt to improve both the level and regulation of WASP protein expression, Dupré *et al*[1] also tested the promoter from the human *WASP* gene in their vector and showed that it expresses quite well. In future, it may be important to add other elements to increase expression, such as those that facilitate RNA processing or serve as a scaffold attachment region.[10] Adding an insulator[10] may serve to protect the vector-encoded gene from the influence of DNA sequences or features of chromatin structure surrounding the integration site.

There is another compelling reason to add an insulator to the vector. A recent, otherwise successful gene therapy trial for severe combined immunodeficiency was complicated by the development of leukemia in two patients.[11] Molecular analysis demonstrated that the leukemia in each patient was due in part to activation of the *LMO2* gene – a known proto-oncogene – by insertion of the oncoretroviral vector genome into or near the *LMO2* gene promoter. The long terminal repeat (LTR) of the oncoretroviral genome contained a powerful promoter and enhancer combination which increased LMO2 expression many-fold.[11]

Recent studies have shown that retroviral integration is far from random.[12,13] For example, mouse viruses preferentially target the region of the transcriptional start site of expressed genes. Although lentiviral vectors lack this predilection, their integration sites are frequently found within expressed genes. The lentiviral vector used by Dupré *et al*[1] is self-inactivating, that is, the final integrated genome has deletions in both of the LTRs which eliminate the promoter and enhancer. Further safety could be achieved by replacing these sequences with an insulator, which would reduce the likelihood of the vector's regulatory elements influencing expression of nearby genes.

Dupré *et al*[1] have focused on introducing the *WASP* gene into T-lymphocytes of WAS patients. Using relatively gentle cytokine stimulation, they were able to achieve a high frequency of transduction while preserving an immunologically naive phenotype that would allow gene-corrected cells to react to specific antigens once reinfused into the patient. The proportion of gene-corrected lymphocytes increased over time in culture, indicating that WASP-expressing cells have a proliferative advantage. Correction of cytokine-secretory defects and augmented formation of lipid rafts reflecting the immunological synapse were also demonstrated in gene-corrected T-lymphocytes from WAS patients.

Could T-lymphocytes serve as a target for therapeutic gene transfer? Certainly, they can readily be obtained from a patient and expanded in culture to derive large numbers of gene-corrected cells. However, all cells of the blood, including macrophages, dendritic cells, neutrophils,

and platelets, are defective in WAS patients. Furthermore, an experiment of nature has taught us that gene correction of T-lymphocytes does not fully eliminate the manifestations of WAS. Rare patients with a germline mutation in the *WASP* gene have a correcting somatic mutation in a T-lymphocyte progenitor that restores expression of WASP.[14] Spontaneously corrected lymphocytes accumulate over time. Although clinical improvement has occurred in some patients, others continue to manifest their disease. Infusing a gene-corrected, autologous T-lymphocyte population may be of some benefit, but there is reason to believe that it would not fully correct WAS.

Overall, this paper reports significant advances in the effort to develop gene therapy for WAS. Careful testing of candidate clinical vectors in the murine models as suggested by Dupré *et al*[1] is mandatory and should provide a firm basis for predicting their potential clinical benefit. Stem cells rather than T-lymphocytes are likely to be the preferred targets of therapy for WAS. ■

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