inside blood commentary

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GENE THERAPY

Comment on Girard-Gagnepain et al, page 1221

Envelope, please. And the award goes to…

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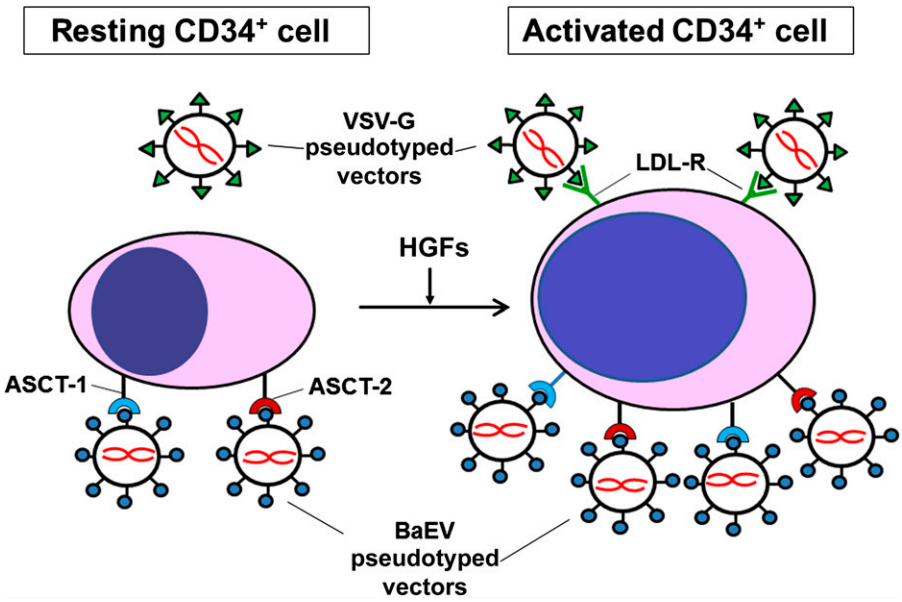
In this issue of *Blood*, Girard-Gagnepain et al report a potentially important discovery for improved gene transfer to human hematopoietic stem cells (HSCs) for gene therapy of blood cell diseases: a novel retroviral envelope to package (pseudotype) vectors for enhanced gene delivery to human HSCs.[1]

Autologous transplantation using gene-corrected HSCs is achieving a growing number of clinical successes for the treatment of primary immune deficiencies, lysosomal storage and metabolic disorders, and hemoglobinopathies.[2,3] Despite the gratifying progress, it remains challenging to achieve optimal gene transfer to human HSCs, and 10- to 100-fold higher amounts of vectors are typically needed for their gene modification compared with transduction of cell lines, mesenchymal stem cells, and many other target cell types. This is perhaps most relevant to efforts toward gene therapy of hemoglobinopathies, where the large, complex human β-globin gene cassettes needed for high-level, erythroid-specific expression lead to lower vector titers and limit gene transfer efficacy, in contrast to what can be achieved with vectors carrying simple cDNA and small promoter elements.



Transduction of human CD34$^+$ HSCs by pseudotyped vectors. Resting CD34$^+$ cells display the ASCT-1 and ASCT-2 proteins that serve as receptors for the BaEV envelope glycoprotein, and retroviral or lentiviral vectors pseudotyped with the BaEV envelope can bind to the receptors and transduce the cells. Resting CD34$^+$ cells lack expression of the LDL-R that serves as the receptor for VSV-G-pseudotyped vectors and thus are not effectively transduced by them. Activated CD34$^+$ cells, prestimulated with HGFs, such as *ckit* ligand, flt-3-ligand, and thrombopoietin, express the LDL-R and can bind and be transduced by VSV-G-pseudotyped vectors. The levels of the ASCT-1 and ASCT-2 receptors increase further in activated CD34$^+$ cells, and transduction by the BaEV-pseudotyped vectors also increases.

The relative restriction of gene transfer to human HSCs has been known for a long time, requiring activation of HSCs for effective transduction,[4] typically achieved by a prestimulation culture period of 24 to 48 hours in medium with a mixture of early-acting hematopoietic growth factors (HGFs), such as *ckit* ligand, flt-3 ligand, and thrombopoietin, prior to vector exposure. The mechanisms by which prestimulation increases gene transfer have been attributed to moving the largely quiescent, G_0 phase HSCs to more active states, allowing higher levels of vector genome reverse transcription. Prestimulation induces stem cell mitosis to allow γ-retroviral vectors to access the chromosomes, because they lack the nuclear import signals present in lentiviral vectors, which lets the latter transduce nondividing cells and largely supplant the use of γ-retroviral vectors. More recently, blocks to transduction by retroviral restriction factors, innate intracellular inflammatory response pathways, or endosomal trapping have been identified as potential roadblocks, with reports suggesting they may be at least partially surmounted with proteasome inhibitors, rapamycin, or other small molecules. Always, the manipulations used to augment gene transfer efficiency (eg, extended culture with HGFs) need to be balanced with the potentially adverse effects from these approaches on HSC capacity, with induction of differentiation likely to limit the longevity of engraftment of the gene-corrected cells.

Part of the search for methods for improved transduction has involved assessments of

different envelopes to pseudotype the vectors for optimal target HSC binding and entry. Initially, envelopes from retroviruses were used that conferred tropism to human cells, such as the murine retrovirus amphotropic envelope, the gibbon ape leukemia virus envelope, or the feline leukemia virus RD114 envelope. The envelope glycoproteins are essentially nontoxic, which allows them to be incorporated into stable "packaging cell lines," and these pseudotypes have been most commonly used for γ-retroviral vectors in clinical trials. However, the use of vectors with these envelope pseudotypes requires surface expression of the specific cellular protein(s) that they exploit as receptors on target cells, typically amino acid or mineral transporters that may require cell activation to be induced.

The vesicular stomatitis virus (VSV-G) protein was identified as having some advantages over retroviral envelopes for pseudotyping retroviral and lentiviral vectors, including the wide species tropism it confers (from human down to zebrafish). The physical hardiness of VSV-G–pseudotyped virion allows vector concentration to high titers without significant loss of biological activity (in contrast to retroviral envelope proteins that are thought to be more fragile, leading to their loss during ultracentrifugation).[5] However, there has been a love-hate relationship with the VSV-G envelope, as it is moderately fusogenic; this causes cytotoxicity and makes it difficult to derive stable packaging lines that produce VSV-G pseudotyped virion, necessitating vector production by cumbersome transient transfection methods (although a first vector produced from a stable VSV-G packaging cell line has entered clinical trials).[6] Also, VSV-G-pseudotyped vectors can be cytotoxic to their target cells, such as HSCs, especially when used at high concentrations or with impure preparations.

Investigators in Lyon, France, reported earlier this year that the VSV-G cellular receptor, only recently identified as the low-density lipoprotein receptor (LDL-R),[7] is minimally expressed on human HSCs; induction of LDL-R expression turns out to be one of the major mechanisms by which prestimulation with growth factors augments HSC transduction.[8] They now report studies using a novel envelope pseudotype derived from the baboon endogenous retrovirus (BaEV) that appears to present an important advance for the production of vectors for gene transfer to human HSCs.[1] Vectors packaged with the BaEV envelope were shown to use 2 cellular proteins, the neutral amino acid transporters ASCT-1 and ASCT-2, as receptors, and basal expression of these receptors on freshly isolated human HSCs allowed moderately effective gene transfer without prestimulation, in contrast to the strict requirement for prestimulation for transduction by VSV-G-pseudotyped vectors (see figure). Although the titers measured for the BaEV-pseudotyped vectors were ~20-fold lower than the VSV-G pseudotypes, they were more effective when used at equivalent amounts (adjusted for virion p24 content). Using both in vitro and in vivo serial xenografts in NOD/SCID/gammaC(null) (NSG) mice as assays of human HSC transduction, the BaEV-pseudotyped vectors led to significantly higher levels of gene transfer to human HSC than VSV-G-pseudotyped vectors: up to 30% of NSG-engrafting cells without prestimulation and up to 90% with prestimulation, with vector present in all lineages of human blood cells produced. Effective transduction could be achieved using fewer HGFs than are typically used, which may contribute to better preservation of stem cell function.

Of course, the xenograft models are still surrogates for the long-term reconstituting HSCs in clinical transplants, and additional studies will need to be performed to verify and extend these findings, both in nonhuman primate models (the BaEV packaged vectors were shown to effectively transduce macaque $CD34^+$ cells) and eventually in clinical trials. However, the identification of the unique properties of the BaEV envelope holds the prospect of improving gene production methods, by allowing the development of stable packaging cell lines, as well as increasing the efficiency of gene transfer to stem cells while better preserving their function by minimizing ex vivo manipulation. This newly characterized envelope may be the award winner for Best Envelope in a Pseudotyping Role needed to take HSC gene therapy to the next level of effectiveness.

Conflict-of-interest disclosure: The authors declare no competing financial interests. ■

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Comment on Lopez et al, page 1232

Hematopoietic ontogeny in the axolotl

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In this issue of *Blood*, Lopez et al undertake the heroic task of characterizing the blood-forming system of the axolotl (*Ambystoma mexicanum*), an aquatic salamander that provides an excellent model for tissue regeneration and scar-free wound healing.[1] Commonly referred to as the "Mexican walking fish," axolotls are not fish at all, but rather neotenic salamanders that retain many larval traits throughout their lifespan because they do not undergo a typical juvenile to adult metamorphosis. This retention of larval traits is associated with the profound ability of the axolotl to regenerate many of its tissues, including limbs, spinal cord, heart, and even parts of its brain.[2-6]