Readers of Arteriosclerosis, Thrombosis, and Vascular Biology are already well aware of the high level of interest in gene therapy as a potential means of combating cardiac and vascular disease. During the last 10 years or so there have been clear demonstrations in a variety of animal models that it is possible to modulate the progress of atherosclerotic vascular disease, restenotic arterial disease, or venous graft disease by transgenic or knockout approaches. These studies have concomitantly generated a series of experiments in which local gene therapy, predominantly using adenoviral vectors, has been used to attempt to ameliorate restenosis in grafts or after vascular injury and to enhance angiogenesis in and around areas of ischemic or infarcted tissue. The essential vision of successful human gene therapy for cardiovascular diseases remains undimmed, but the original enthusiastic optimism has been tempered by reality: there are significant generic problems such as inflammatory side effects due to the current generation of viral vectors and potential safety concerns for long-term gene therapy highlighted by the cases of leukemia in children being treated for immunodeficiency diseases in addition to the complications inherent in trying to treat complex disease processes with a single magic bullet. Nonetheless, several uncontrolled clinical trials, but very few controlled trials so far, have suggested benefit from local transfer of the gene for vascular endothelial growth factor into the heart after myocardial infarction or into the leg to overcome peripheral ischemia.

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While the significant hurdles that need to be overcome to make cardiovascular gene therapy a clinical reality have become more apparent, the dramatic discovery that progenitor cells derived from bone marrow can make an important contribution to endothelial cells in new or repairing adult blood vessels (with the corresponding, but less certain, recognition that bone-marrow–derived precursors may also generate vascular smooth muscle cells and even cardiac myocytes) has led to a remarkably rapid translation from animal models to human clinical studies in which autologous endothelial progenitor cells (EPCs) derived from blood or bone marrow have been locally injected or implanted to encourage neoangiogenesis in infarcted or ischemic tissues. So far these procedures are apparently safe, and there have been claims of clinical benefit, though there are as yet no completed controlled trials and the exact mechanisms by which benefit may be obtained are still not fully understood. The huge growth of interest in progenitor cell biology, coupled with advances in basic stem cell biology, has recently led to proposals to combine autologous progenitor cell therapy with ex vivo genetic manipulation of these cells and selection and expansion of cell populations with desirable attributes to enhance their clinical efficacy.

By contrast with the complex pathophysiology of cardiovascular diseases, the monogenic blood clotting disorders, including hemophilia, have long been recognized as favorable diseases in which to test whether gene therapy can lead to a cure: A single defined protein deficiency causes the disease; usually relatively inefficient replacement (to <10% of normal levels) is adequate; there is evidence over many years that recombinant proteins are clinically effective; and replacement does not require targeting as the proteins are active in the circulation. The first human trials of gene therapy for hemophilia started 5 years ago, following convincing data in animal studies that the disease could be cured for a period of up to several years following viral or nonviral gene transfer. So far, a handful of phase I trials in humans have suggested that in vivo viral gene transfer can be safe and may be effective. However, there is as yet—at least in part due to the technical complexity of in vivo gene therapy by comparison with administration of recombinant protein—only tentative progress toward phase II/III trials.

Against this background, the article by Herder et al in this issue of Arteriosclerosis, Thrombosis, and Vascular Biology provides a significant step forward in approaches to hemophilia gene therapy and also has direct relevance to the use of EPCs for gene therapy in cardiovascular diseases. One of the initial clinical trials of gene therapy for hemophilia A used the strategy of ex vivo somatic cell gene transfection. Roth et al grew dermal fibroblasts from patient biopsies, selected for factor VIII (FVIII)-producing cells after nonviral gene transfer, propagated these cells, and implanted them into the omentum. In 4 of the 6 patients, FVIII levels rose transiently, with apparent clinical benefit. More striking proof of the principle of this approach was provided last year by Lin et al, who cultured human EPCs from peripheral blood, nonvirally transfected them with the FVIII gene, and selected and expanded these cells ex vivo. Relatively low cell numbers (5 to 40 x 10⁶) were injected intravenously into non-obese diabetic/severe combined-immunodeficient mice, which showed sustained high levels of human FVIII expression over several months before the experiment was terminated. Cells retaining endothelial phenotype and transgene expression...
were found in the bone marrow and spleen. At around the same time, the research group responsible for the current paper was evaluating the ability of a variety of hematopoietic cell lines to stably secrete FVIII after lentiviral transduction,17 noting that cells of erythroid or megakaryocytic lineage could be induced to produce reasonable levels of FVIII. Lentiviral constructs have several potential advantages for clinical use, including long-term expression in nondividing cells, and are being actively investigated for several therapeutic indications.18

In the current paper, Herder et al have combined the use of cord-blood–derived EPCs with lentiviral transduction and successfully generated and expanded cell lines that stably secreted high levels of FVIII throughout the studied in vitro growth period of up to 80 days. These cells retained expression of several endothelial cell marker molecules, which was not detectably altered from nontransduced cells. Transduction efficiency was high (80 to 90%). Furthermore, the level of FVIII secretion per cell was 10- to 20-fold higher than they achieved by the same method with the hematopoietic cell lines.17 The likely superiority of lentiviral transduction over retroviral transduction was also shown by noting that they could achieve comparably high secretion of FVIII from lentivirally transduced human umbilical vein endothelial cells (HUVECs), whereas others had reported 10-fold lower secretion following transduction of HUVECs with a retroviral FVIII construct.19

These results, together with those of Lin et al,16 give strong support for the idea that EPCs can be genetically manipulated ex vivo, expanded, and reintroduced in vivo, where at least a proportion will contribute to a long-lasting pool that can provide therapeutically relevant levels of transgene expression. Further in vivo experiments are obviously needed to test this idea thoroughly. Whether this strategy will become clinically important for the treatment of hemophilia is not yet clear, though it is a tantalizing prospect. The hope that therapy may be long-lasting gives a substantial theoretical advantage over treatment with recombinant proteins, and the expanded populations of autologous cells can be viably stored for future repeated use if needed.

The results have two additional general implications that enhance the prospect that EPCs could be clinically valuable in a wide variety of diseases. First, they demonstrate that EPCs can readily be transduced with lentiviral constructs with the expectation of stable long-term expression of the transgene, a property that could be exploited equally well when EPCs are locally delivered to encourage angiogenesis or vascular repair. Second, they open up the prospect that EPCs can be used to provide a depot of autologous cells for the ectopic synthesis and secretion of any one of a wide variety of proteins with systemic effects via the circulation. There will undoubtedly be setbacks before the uses envisaged here become a clinical reality, but by combining to advantage two topical aspects of cell and molecular biology, Herder et al have put another necessary intermediate step in place.

References
