Gene delivery goes global

Clare E Thomas

For gene therapy of muscular dystrophies, all of the skeletal muscles in the body must be transduced—a tough challenge. An approach that permeabilizes blood vessels using VEGF, helping low doses of a parvovirus-based gene therapy vector sneak across the vessel wall, now solves this problem in mice (pages 828–834).

The concept sounds simple: viruses are adept at delivering their own genes into human tissues, so replace the most harmful viral genes with a therapeutic gene and the pathogen becomes a medicine. But translating the concept of gene therapy into practice has been fraught with obstacles, many expected and some unforeseen. A particularly challenging problem has been achieving widespread transgene expression in skeletal and cardiac muscle—a prerequisite for gene therapy of disorders such as the muscular dystrophies. Delivery through the circulation would seem to be an attractive option for targeting these tissues, but the vascular endothelium presents an impenetrable barrier to most gene therapy vectors.

Writing in this issue[1], Gregorevic and Blankenship *et al.* find a solution to this problem. They inject a protein that makes vessels transiently permeable—vascular endothelial growth factor (VEGF)—into the bloodstream of mice, together with a viral vector based on adeno-associated virus type 6, and thereby achieve efficient whole-body transduction of skeletal and cardiac muscle.

Wild-type viruses have evolved to efficiently transfer their genome into cells—and to replicate efficiently once inside. Their vector counterparts, however, are usually disabled so that they cannot replicate inside the cells they infect. But this safety feature has an unfortunate consequence. Inject most classes of replication-defective viral vector directly into tissue and the vector particles don't diffuse far: cells closest to the injection site suck up the virus like a sponge, or else the virus particles get stuck in the extracellular matrix.

Various attempts have been made to increase the biodistribution of proteins expressed by gene therapy vectors. One strategy has involved tagging the therapeutic proteins with a peptide—the protein-transduction domain from HIV Tat—which enables them to be exported from one cell and then taken up by its neighbors. This approach has boosted the distribution of β-glucuronidase (missing in the lysosomal storage disorder mucopolysaccharidosis type VII) after lentiviral delivery of this transgene to the brains of mice[2]. But to achieve functional correction of disorders affecting the heart and all the skeletal muscles, the best approach remains to find a way to deliver the viruses through the circulation.

Progress in this area has so far been limited to performing complicated and invasive surgical techniques (involving, for example, temporarily clamping the local blood supply to the tissue after intravenous administration) or increasing vessel leakiness using the inflammatory mediator histamine. None of these procedures has yet resulted in efficient whole-body gene expression in muscle tissue. Gregorevic *et al* now take matters a step further, by exploiting a parvovirus-based gene therapy vector together with a molecule best known for regulating the formation of new blood vessels—VEGF. VEGF was originally described as a 'vascular permeability factor'. By influencing the morphology of the endothelium, VEGF can also affect the leakiness of the blood-vessel wall.

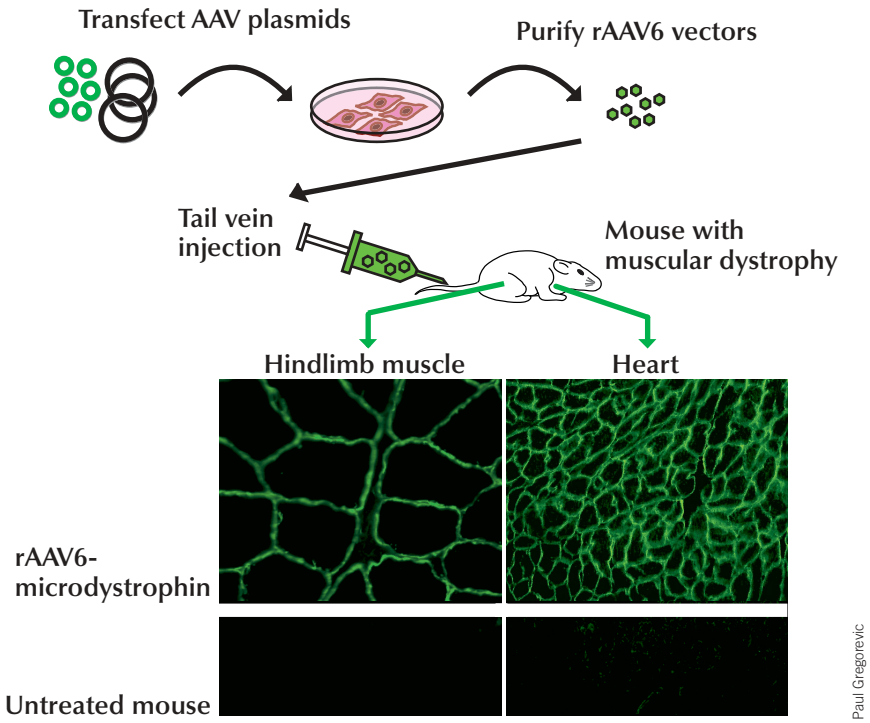

Figure 1 Delivery of a therapeutic gene to the skeletal muscles and heart of an adult animal with muscular dystrophy. The delivery scheme of Gregorevic and Blankenship *et al.* uses a recombinant AAV serotype 6 vector (rAAV6), which is produced by plasmid transfection of cell cultures without the need of a helper virus. Purified rAAV6 vectors carrying a highly functional 'microdystrophin' gene are injected into the tail vein of adult mdx mice, which lack dystrophin and models Duchenne muscular dystrophy. A single injection of vector, using a promoter that is active in all striated muscles, restores dystrophin production in the heart, limb and respiratory muscles (identified by green immunolabeling) and leads to a whole-body amelioration of the dystrophic pathology.

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AAV6, the virus the authors used in conjunction with VEGF, is a member of an ever-expanding family of parvoviruses that are being developed as vectors. Eight AAV serotypes have so far been developed as gene delivery vehicles[3], each with different abilities to infect various tissues. AAV2 has been most widely used in gene therapy applications so far, but AAV6 is emerging as a promising alternative for transducing certain tissues, notably the lungs and skeletal muscle.

Starting with AAV6 particles expressing β-galactosidase as a means to visualize the extent of transduction, the authors injected the viruses into the tail veins of mice. A moderate dose of virus alone yielded unimpressive results, but coadministration of VEGF boosted transduction of skeletal muscles as much as 100-fold. Almost all cardiomyocytes and skeletal muscle cells throughout the body expressed β-galactosidase after systemic administration of high doses of virus in conjunction with VEGF. Curiously, at these high doses, the virus was able to cross the vascular endothelium and efficiently transduce muscle even in the absence of VEGF.

The authors also report remarkable global reconstitution of dystrophin expression in the *mdx* mouse model of Duchenne muscular dystrophy (**Fig. 1**). After systemic VEGF-mediated delivery of an AAV6 vector expressing 'micro-dystrophin' (a highly truncated yet functional dystrophin coding sequence) under the control of a muscle-specific promoter, the authors found dystrophin expression throughout the skeletal muscles of mice, with partial phenotypic correction—the serum of treated mice contained less creatine kinase (an enzyme marker indicative of muscle degeneration) and their limb muscles were less susceptible to injury.

But is the procedure safe and practical for use in humans? VEGF has been administered systemically to humans and its effects on blood vessels seem to be short-lived. Mice tolerated treatment with AAV6 and VEGF well—acute organ toxicity was not seen. But vector genomes were detected in many other tissues beside the skeletal muscle, including the brain and testes, and this broad tropism of AAV6 might be considered undesirable, despite the ability to restrict transgene expression with cell-type specific promoters.

Whole-body muscle transduction after systemic delivery of AAV6 and VEGF brings us a step further toward gene therapy for skeletal muscle and cardiac disorders, but some key questions remain. For example, how, after injection of high doses, is AAV6 able to traverse the vascular endothelium in the absence of a permeabilizing agent? We know relatively little about the mechanisms of transduction of AAV6 as compared with the more widely used AAV2. The primary cellular receptors for AAV2 and AAV5 have been identified—heparin sulfate proteoglycans (HSPG) and PDGF, respectively[4,5]—but all we know about the receptor for AAV6 is that it is not HSPG[6].

So would the VEGF approach described by Gregorevic *et al.* work using other viruses? Perhaps. Indeed, in a previous study, Greelish *et al.*[7] showed that transduction of hindlimb muscle after intravenous administration of adenovirus vectors could be boosted by inducing vascular permeability with a combination of papaverine (a vasodilator) and histamine. But adenoviruses are not ideal vectors for systemic delivery, as the adenovirus capsid is a potent trigger of the inflammatory response. AAV, in contrast, is noninflammatory. And importantly, AAV-based vectors are able to sustain long-term transgene expression in nondividing cells.

Notwithstanding these advantages, there is at least one serious limitation to the use of AAV as a gene therapy vector—AAV particles are tiny and can package less than 5 kb of DNA. But as the authors point out, the utility of this systemic delivery technique is likely to extend beyond therapeutic applications; the approach could be coupled with RNA interference, or Cre-recombinase technology, and used to produce new animal models for experimental research.

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Type 1 diabetes: focus on prevention

Harald von Boehmer

Two new approaches prevent disease in a model of type 1 diabetes. One approach blocks an activation receptor on disease-conferring T cells. The second deploys suppressor T cells renowned for their ability to inhibit the local immune response.

During type 1 diabetes, $CD4^+$ and CD8 T^+ cells contribute to the destruction of insulin-producing β-cells in the pancreas. Recent studies outline two approaches to combat this destruction. In the June issue of *Immunity*, Ogasawara *et al.*[1] show that antibodies blocking NKG2D, a costimulatory receptor on activated $CD8^+$ T cells, can prevent type 1 diabetes in a mouse model. In the June 7 issue of *The Journal of Experimental Medicine*, two studies[2,3] harness a protective type of T cell, the suppressor T cell, to modulate immune responses—providing proof of principle that expansion of antigen-specific suppressor cells can be used as a tool to interfere with development of type 1 diabetes.

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Type 1 diabetes–causing $CD4^+$ and $CD8^+$ T cells are activated by antigen released from pancreatic islet cells that contain β-cells. The disease process begins with infiltration of mononuclear cells around the islets and proceeds with destruction of insulin-producing β-cells to the point that an exogenous supply of insulin becomes mandatory to sustain life.

The most extensively studied animal model of human disease is the nonobese diabetic (NOD) mouse. This mouse strain spontaneously develops diabetes, in part owing to the activity of $CD8^+$ T cells. Ogasawara *et al.*[1] show that in NOD mice, activated $CD8^+$ T cells infiltrating the pancreas express a costimulatory NKG2D receptor in addition to the T-cell receptor for antigen (**Fig. 1**). The ligand of the NKG2D receptor, RAE-1, is expressed on non-hemopoietic pancreatic tissue in the prediabetic NOD strain but not in the nondiabetic BALB/c strain of mice. The authors observed that continuous injection of a blocking NKG2D-specific antibody from seven weeks of age onwards interfered