Gene Therapy: the Ball Takes a Spin towards Multifactorial Diseases

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The first clinical trial in gene therapy was initiated almost 10 years ago (September 1990). Since then, over 300 such trials have been conducted, and more than 3,000 patients are carrying genetically engineered cells in their body [1]. Nevertheless, somatic cell gene therapy is only now exerting its impelling force onto multifactorial diseases, such as ischemic vascular diseases (particularly of the heart), cancer, and autoimmune diseases.

Two review articles in the current issue of *IMAJ* present an updated review of gene therapy treatment in ischemic vascular diseases [2,3] and cancer. For the treatment of ischemic vascular diseases, vascular endothelial cell growth factor has been selected as a promising candidate for gene therapy since it stimulates (*in vivo*) capillary formation and increases vascular permeability. The effect appears to be limited to endothelial cells. Most importantly, VEGF receptors are upregulated upon hypoxia or ischemia. Thus, VEGF has a minimal effect on normal endothelium *in vivo*. This makes VEGF gene a very good candidate for gene therapy in ischemic vascular diseases, particularly of the heart [4,5]. In cancer gene therapy the idea of delivering genes capable of inducing cellular death or suppressing tumor cell growth looks promising, but is still far from being a magic bullet [6,7].

Conceptually, gene therapy has been used as an efficient methodology to circumvent genetic deficiency by transfection of cDNA encoding the appropriate functional gene product. It is therefore conceivable that the best candidates for this mode of therapy would be genetic diseases associated with a single gene mutation, such as X-linked agammaglobulinemia or cystic fibrosis. Paradoxically, it appears that gene therapy needs to confront similar levels of technological challenges when encountering genetic disorders, such as XLA or CF, to those required for a successful intervention in multifactorial diseases. Yet, while genetic disorders that evolve a mutation in a single gene are rare, multifactorial diseases are a major cause of illness and death in the developed countries. This has motivated scientists to explore gene therapy strategies in multifactorial disorders. Among the multifactorial diseases, ischemic vascular diseases and cancer are the major cause of death in the western world and are therefore attractive candidates for gene therapy.

A major technological challenge in gene therapy has been the development of an efficient and safe drug delivery system. During evolution the human body evolved to protect itself from the onslaught of hazards, including incorporation of foreign DNA into the genome. Some viruses, however, are successful in overcoming these barriers and are able to insert their genetic material into human cells. Hence, initial efforts in gene therapy were directed towards engineering viral vectors to carry genes into human patients. The establishment of effective therapy using vectors for gene delivery must meet four important technical challenges. These are:

- The need to target each gene to the appropriate cells in the relevant target organ.
- The ability to induce either stable or transient transfection of genes, depending on the specific strategy chosen for each disease.
- The necessity to control the production of each gene product (particularly in stable transfections).
- Safety.

The first two requirements are still major limiting factors for the successful utilization of gene therapy in various diseases [1,8–10]. The most successful means of achieving stable expression is *ex vivo* treatment of cells. Such an approach however, requires co-transfection of an antibiotic resistance gene and a selection process [1,9]. Recently, the gene encoding green fluorescent protein has also become a useful selection marker for positively transfected cells. Naturally, this type of therapy can be applied to blood cells and their precursors since these cells are the easiest to remove and return. The second approach is *in situ*, where the vector is placed directly into the affected tissue. Examples include the infusion of adenoviral vectors into the trachea and bronchi of patients with CF, the injection of tumor mass with a vector carrying a dystrophin gene directly into the muscle of a patient with muscular dystrophy, and arterial gene transfer using a naked DNA plasmid encoding VEGF into the vessel

VEGF = vascular endothelial growth factor
XLA = X-linked agammaglobulinemia
CF = cystic fibrosis

wall at the time of balloon inflation [4,5]. The third therapeutic route includes the design of a vector capable of targeting to a specific organ/tissue/cell that can be directly injected into the circulation. This approach has recently been explored in cancer therapy. The basic idea is to use these vectors to deliver genes capable of inducing cellular death or suppressing the growth of tumor cells (reviewed in the current issue by Zisman et al.). However, a very recent publication in the last issue of *Nature Medicine* shows that such a therapy could also be problematic, and suggested that treating glioma with an adenoviral mediated suicide gene may lead to bystander damage to other cells in close proximity and the development of an inflammatory process at the target organ [6,7].

Except for cardiovascular diseases and cancer, autoimmune diseases have recently become a major field of interest for gene therapy. Two different novel strategies have recently been suggested for the therapy of T cell-mediated autoimmune diseases. The first approach involves *in vitro* transfection of antigen-specific autoimmune T cells with a regulatory gene of interest, such as interleukin-4 or -10. Upon injecting the manipulated T cells into the circulation, the cells are expected to home in to the target autoimmune organ and propagate the response to the specific autoimmune determinate. While so doing they produce and secrete the desired regulatory gene product at this site and restrain the relevant autoimmune disease [11]. The second approach was recently utilized by my group. We employed a modification of naked DNA vaccination to induce tolerance breakdown to several pro-inflammatory cytokines and chemokines. cDNA encoding each gene of interest was cloned into a mammalian expression vector with a strong viral promoter (cytomegalovirus) and a repeated immunostimulatory sequence [12]. The repeated DNA vaccine administration led to a breakdown of tolerance to the product of each inserted gene and to the generation of long-term immunological memory. Following the initiation of experimental autoimmune encephalomyelitis — a T cell-mediated autoimmune disease of the central nervous system that serves as an experimental model for multiple sclerosis — this memory "turns on" to provide protective immunity [13,14]. The protective competence can be adoptively transferred by self-specific neutralizing antibodies. Surprisingly, elicited production of these antibodies was found to be dependent on the development of an autoimmune condition and is regulated by the immune system in accordance with disease progression [13,14]. This could provide the immune system of a patient suffering from an autoimmune condition with a powerful tool to restrain its own harmful activities [13,14].

In conclusion, at the threshold of the new millennium, molecular medicine is beginning to exert its impelling force onto multifactorial diseases, which are a major cause of chronic illness and death in developed countries Three years ago, in his *Nature Medicine* commentary, T. Friedmann concluded that for "human gene therapy — the genie is immature, but certainly out of the bottle" [8]. Well, as things are moving now the genie will be reasonably mature before his Barmitzvah.

References

1. Anderson WF. Human gene therapy. *Nature* 1998;392(6679 Suppl):25–30.
2. Baumgartner I, Isner JM. Gene therapy for peripheral vascular disease. *IMAJ* 2000;2:27–32.
3. Zisman A, Pantuck AJ, Belldegrun A. Gene and immune-based therapies for genitourinary malignancies: current status and future prospects. *IMAJ* 2000;2:33–42.
4. Lee JS, Feldman M. Gene therapy for therapeutic mycocrdial angiogenesis: A promising synthesis of two emerging technologies. *Nat Med* 1998; 4(6):739–42.
5. Ferrara N, Alitalo K. Clinical applications of angiogenic growth factors. *Nat Med* 1999;5(12):1359–64.
6. Dewev RA, Morrissey G, Cowsill CM, Stone D, Bolognani F, Doddo NJF, Southgate TD, Klatzmann D, Lassmann H, Castro MG, Lowenstein PR. Chronic brain inflammation and persistent herpes simplex virus 1 thymidine kinase expression in survivors of syngeneic glioma treated by adenovirus mediated gene therapy. *Nat Med* 1999;5:1256–63.
7. Kiellan T, Hickey WF. Inflammatory thoughts about glioma gene therapy. *Nat Med* 1999;11:1237–8.
8. Friedmann T. Human gene therapy — an immature genie, but certainly out of the bottle. *Nat Med* 1996;2:144–7.
9. Gage FH. Cell therapy. *Nature* 1998;392(6679 Suppl):18–24.
10. Verma IM, Somia N. Gene therapy — promises, problems and prospects [News]. *Nature* 1997;389(6648):239–42.
11. Mathisen PM, Tuohy VK. Gene therapy in treatment of autoimmune diseases. *Immunol Today* 1998;19(3):193–5.
12. Sato Y, Roman M, Tighe H, Lee D, Corr M, Nguyen M, Silverman GJ, Lotz M, Carson DA, Raz E. Immunostimulatory DNA sequences necessary for effective intradermal gene immunization. *Science* 1996;273:352–7.
13. Wildbaum G, Karin N. Augmentation of natural immunity to a pro-inflammatory cytokine (TNF-α) by targeted DNA vaccine confers long lasting resistance to experimental autoimmune encephalomyelitis. *Gene Ther* 1999;6:1128–38.
14. Youssef S, Wildbaum G, Maor G, Lanir N, Gour-Lavie A, Grabie N, Karin N. Long lasting protective immunity to experimental autoimmune encephalomyelitis following vaccination with naked DNA encoding C-C chemokines. *J Immunol* 1998;161:3870–9.

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The difference between genius and stupidity is that genius has its limits.

Albert Einstein