Editorial

Current status of human gene therapy

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Human gene therapy (HGT) is defined as the transfer of nucleic acids (DNA) to somatic cells of a patient resulting in a therapeutic effect by either correcting genetic defects or over-expressing proteins that are therapeutically useful[1]. In the past, both professional and lay community had high expectations from HGT. However, although the theoretical advantages of HGT are indisputable, so far it has not delivered the promised results.

Foreign genes may be inserted into somatic cells, germ cells or early embryonic cells. Insertion of genetic material into somatic cells and their subsequent transplantation is not fundamentally different from organ transplantation or blood transfusion. Insertion of genes into fertilised eggs or early embryos raises profound ethical issues because these genes would be passed on to the offspring in subsequent generations[2].

The targets of gene therapy are either intracellular or extracellular. In the former, the gene affects the cell itself by replacing a defective or missing gene or providing a product that kills or inhibits the growth of cells. In the latter, the cell releases the product of the new gene which can either act locally on neighbouring cells or enter the circulation for delivery to distant cells[3].

Most gene delivery to date has involved the use of viruses as carriers of the gene. Moloney murine leukaemia retrovirus (MLV) is the first virus to be used as a genetic transfer agent in humans[4]. More clinical trials have been undertaken with MLV than with any other type of vector. MLV lacks virtually all viral genes except those needed to infect mammalian cells. It is so defective that after infecting the target cell, it cannot replicate or infect other cells[3]. Further, as it is easily generated, infected virus can be extensively characterised in tissue culture before injection into patients and stable integration of the virus into the chromosome ensures its retention by the cell. The risk of MLV integration into the cellular genome inactivating host tumour-suppressor genes or activating proto-oncogenes can be minimized by reducing the efficacy of infection so that at most one virus infects a cell[3]. MLV has 2 main limitations. Firstly it can harbour genes of only 7 kilobase (kb) size. Secondly it infects only dividing cells whereas most adult tissues comprise non-dividing cells[5]. Thus MLV is mainly used for ex vivo gene delivery i.e. injection of cells genetically engineered by infection outside the body rather than by in vivo infection by the retrovirus itself[3].

Host cell mitosis is not required by the class of retroviruses known as lentiviruses which include HIV-1. These viruses contain specific proteins that enable the viral RNA genome to be trafficked to the nucleus and enter through nucleopores. Once in the nucleus reverse transcription and integration occur even in the absence of cell proliferation[6].

Adenoviruses, the second commonest viral vectors for gene delivery, readily infect non-dividing cells and can be produced in bulk, making infection of adult tissues more efficient[3]. Further, these viruses remain extra-chromosomal, thus reducing the chance of disrupting the cellular genome. A limitation of adenoviruses is that if delivered in vivo they infect all tissues, including the germ line and thus could affect subsequent generations. Further, they are not as defective as retroviruses and could more readily yield infectious virus in the body[3]. Another problem is that the current generation of adenoviral vectors is immunogenic which reduces the time available for the expression of the gene and makes repeated administration of the vector impossible[3].

Adeno-associated virus (AAV) vectors are capable of high efficiency transduction of both dividing and non-dividing cells and tissues[7]. AAV-mediated transduction leads to stable, long-term transgene expression in the absence of apparent immune response. Major restrictions of AAV as vectors are their limited genetic capacity and strict packaging size constraints of less than 5 kb. Another difficulty is the labour-intensive and expensive procedure for the production and packaging of recombinant AAV vectors[7].

Herpes simplex virus (HSV) is particularly adapted to the establishment of latent infections in sensory neurons and the central nervous system (CNS) which

makes HSV-based vectors potentially useful for the treatment of neurologic diseases[8]. HSV cells have the ability to infect non-dividing cells and also have a large packaging capacity. The primary disadvantage of HSV vectors is the potential for cellular and immunologic toxicity[9].

There are also non-viral methods of gene delivery. Exogenous genes have been successfully delivered to cells in vitro and in vivo by receptor-mediated endocytosis. Receptor-mediated gene transfer affords specificity as a non-infectious vector by exploiting receptors on the cell surface to bind and internalise complexes containing the functional gene. This form of gene transfer allows specific tissue targeting with DNA plasmids of considerable size, permitting the delivery of not only the transgene but also promoter and enhancer elements[9]. Liposomes or lipid vesicles, which combine readily with cell membranes, are being used to deliver genes, sometimes as aerosols[3]. Liposomes are also being tested in conjunction with viruses to enhance localized delivery of genes[3]. A potential limitation in the use of liposomes to mediate gene transfer is their relative lack of specificity[9].

Severe combined immunodeficiency (SCID) caused by adenosine deaminase deficiency (ADA) was the first genetic disorder to be treated with gene therapy[10]. Since 1990 when the first trial started for 2 patients with ADA-SCID, 5 clinical trial enrolling 11 patients have been conducted with different clinical approaches and the results obtained from these trials have been reported. According to these reports T-cell-directed gene transfer was useful in the treatment of ADA-SCID whereas the retroviral-mediated gene transfer to haematopoietic stem cells was insufficient for achievement of clinical benefits[10].

Approaches to gene therapy for the haemophilias include ex vivo gene therapy in which cells from the intended recipients are explanted, genetically modified to secrete Factor VIII or IX and re-implanted into the donor; in vivo gene therapy in which Factor VIII or IX encoding vectors are directly injected into the recipient; and non-autologous gene therapy in which universal cell lines, engineered to secrete Factor VIII or IX, are enclosed in immuno-protective devices before implantation into recipients[11]. Problems of achieving high and sustained levels of factor delivery and issues related to efficacy, safety and cost are still to be resolved[11].

The current principle behind gene therapy in cystic fibrosis (CF) is the insertion of DNA encoding normal CF transmembrane conductance regulator (CFTR) into respiratory cells which should overcome the abnormalities seen in CF[12]. So far, there have been 5 phase I clinical trials using adenovirus as a vector, one using AAV and 4 with liposomes. In these trials approximately half of the patients show molecular evidence of gene transfer and about one third demonstrate a degree of correction of the chloride defect. No trial has demonstrated correction of the sodium hyperabsorption[12].

Gene therapy for muscular dystrophy presents significant challenges, including the large amount of muscle tissue in the body, the large size of many genes defective in different muscular dystrophies and the possibility of a host immune response against the therapeutic gene[13]. Encouraging progress has been made in modifying adenovirus vectors to reduce immune response and increase capacity[13].

The numerous different approaches to gene therapy for treatment of cancer can be subdivided into 3 basic concepts viz. strengthening of the immune response against a tumour, repair of cell cycle defects caused by losses of tumour suppressor genes or inappropriate activation of oncogenes, and suicide gene strategies[14]. So far, there has been a disappointing inability to reach target cells with sufficient efficacy to generate high enough levels of direct killing and this has necessitated the invocation of bystander effects in order for any potential strategy to be convincing[15].

Gene therapy approaches for human immunodeficiency virus (HIV) infection include efforts to interfere with viral replication directly by engineering HIV-resistant cells or indirectly by eliminating infected cells from the body primarily by eliciting a therapeutic immune response to destroy HIV-infected cells[16].

Recent human clinical trials have shown that injection of naked DNA encoding vascular endothelial growth factor (VEGF) promotes collateral vessel development in patients with critical limb ischaemia or chronic myocardial ischaemia[17].

Modification of viral vectors (to reduce immunogenicity, change tropism and increase cloning capacity), engineering of non-viral vectors by mimicking the beneficial properties of viruses, cell-based gene delivery technologies and development of innovative gene expression regulation systems, together with the ever increasing knowledge and experience in the field will undoubtedly lead to the realisation of the full potential of HGT in the future[1].

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