Gene therapy: Principles, practice, problems and prospects

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The remarkable advances made in recombinant DNA technology over the last two decades have paved way for the use of gene transfer to treat human diseases. Several protocols have been developed for the introduction and expression of genes in humans, but the clinical efficacy has not been conclusively demonstrated in any of them. The eventual success of gene therapy for genetic and acquired disorders depends on the development of better gene transfer vectors for sustained, long term expression of foreign genes as well as a better understanding of the pathophysiology of human diseases. It is heartening to note that some of the gene therapy protocols have found other applications such as the genetic immunization or DNA vaccines, which is being heralded as the third vaccine revolution. Gene therapy is yet to become a dream come true, but the light is seen at the end of the tunnel.

THE advent of recombinant DNA technology and the availability of a wide array of gene cloning techniques have had a dramatic impact on the conduct of research in biology and medicine. The molecular biological revolution has opened the floodgates for basic and applied research and researchers are using genes as double-edged weapons for both understanding basic cellular processes as well as biotechnological applications. Techniques such as polymerase chain reaction, non-radioactive nucleic acid probes, ribozymes, transgenics, gene knockouts, chromosome jumping and fluorescent *in situ* hybridization have revolutionized disease diagnosis and therapy. Genetic information in the form of expressed sequence tags (ESTs) and whole genome sequences are filling computer data banks faster than ever and never has gene cloning found more prominence in biology than it has now[1]. Thanks to the brute force approach of positional cloning, it is now possible to identify a diesease gene without any knowledge of its biochemical function. The difference between basic and applied research is disappearing fast and the results of basic research are finding immediate biotechnological applications. Gene cloning has enabled us to link DNA repair and cancer, transcription and xeroderma pigmentosum, cell cycle and malignancy and glycolysis and Huntington's disease. Thus, genes are the ultimate molecular switches that control various cellular processes and abnormal gene expression can manifest in the form of specific genetic disorders and even compromise the ability of an individual to destroy invading pathogens and tumour cells. It is this overwhelming evidence in favour of genes and their critical role in maintaining physiological homeostasis that has strengthened the notion of using genes as therapeutic agents. The idea of delivering genes into humans to correct a disease phenotype remained a biologist's fantasy for a long time until the last decade, when it has been accepted as a scientifically viable proposition, recognized as an independent discipline and christened 'gene therapy'.

Principles and practice

The ultimate goal of human gene therapy is to replace a defective gene with a normal one via targeted insertion into the genome by homologous recombination. Such a strategy, referred to as gene replacement therapy, would not only permit physiological regulation of the transgene but also eliminate the possibility of insertional inactivation of other cellular genes, which happens during random integration of the foreign gene. However, the frequency of random integration versus site-specific integration is so overwhelmingly in favour of the former that gene replacement therapy continues to elude biologists. Recent studies with adeno-associated viral vectors have demonstrated that it is possible to deliver genes to a specific locus in human chromosome 19 and this is an improvement over random integration of genes[2]. A major advantage of integrating foreign genes into the genome is that the expression is relatively stable and long-lasting and in case of dividing cells, the gene is acquired by the daughter cells as well. An alternative approach has been to avoid genome integration altogether and express genes transiently. However, in this case, the foreign DNA is more susceptible to nuclease degradation, and the gene expression is not as stable as seen in integrated genes. Transient vector systems are appropriate for therapies requiring short-term expression, such as in cancer therapy protocols but is not favoured for long-term correction of genetic defects. Overall, the current strategy for gene therapy largely centres around gene augmentation therapy, wherein the foreign gene

replaces the product of the defective or missing gene, but does not physically replace the defective gene itself. At present, two gene transfer strategies are in vogue: the *in vivo* approach, which involves introducing genes directly into the target organs of an individual whereas in the alternative *ex vivo* approach, cells are isolated for gene transfer *in vitro*, followed by transplantation of genetically modified cells back into the patients[3].

All the genetic manipulations that can be performed in humans can be classified into four distinct categories: i) somatic gene therapy (correction of a genetic defect in the somatic cells of the body), ii) germ-line gene therapy (introduction of genes into the germ cells for the correction of the genetic defect in the offsprings), iii) enhancement genetic engineering (gene transfer for improvement of a specific trait, such as introducing a growth hormone gene to increase height), iv) eugenic genetic engineering (genes may be inserted to alter or improve complex traits such as intelligence and personality). While somatic gene therapy is now being attempted on human patients, germ-line gene therapy and enhancement genetic engineering are being carried out in laboratory and farm animals. Germ-line gene therapy is not being attempted in humans for reasons that are as much ethical as technical and eugenic gene therapy is far beyond our technical capabilities and ethically questionable. In fact, the technical problems associated with somatic gene therapy in humans are so overwhelming, that unless we gain sufficient expertise in performing successfully gene transfer in the somatic cells, it may not be possible to carry out other genetic manipulations in humans[4].

Somatic gene therapy was initially formulated for the treatment of monogenic defects, but now holds promise for a wide range of disorders including cancer, neurological disorders, heart disease and infectious diseases (Table 1). A variety of gene delivery strategies have been developed in the last decade for the treatment of human diseases and these can be grouped in two major categories: the viral and non-viral methods. Both these approaches are being pursued vigorously, although gene transfer through the viral vectors is more efficient than that by non-viral methods. Over several million years, viruses have evolved efficient mechanisms for the delivery of their own genomes into cells they infect and the strategy has been to replace these genes with those

Table 1. Genetic and acquired diseases amenable to gene therapy*

Disease	Therapeutic gene	Strategy	Vector	Target cells/tissue
Genetic disorders				
Cystic fibrosis	CFTR	*In vivo*	Adenovirus	Nasal epithelium
		In vivo	Cationic lipids	Nasal epithelium
Familial hyper cholesterolaemia	LDL receptor	*Ex vivo*	Retrovirus	Hepatocytes
SCID	ADA	*Ex vivo*	Retrovirus	T cells, $CD34^+$ stem cells
Haemophilia	Factor VIII/Factor IX	*In vivo*	Retrovirus	Hepatocytes, skin, muscle
		Ex vivo	Retrovirus	Hepatocytes, myoblasts
DMD	Dystrophin	*In vivo*	Retrovirus	Skeletal muscle
		Ex vivo	Retrovirus	Myoblasts
Acquired disorders				
Cancer	Interleukins, HSV-TK, TNF, HLA-B4, tumour suppressor genes	*Ex vivo*	Retrovirus	Tumour cells
		In vivo	Cationic lipids	Tumour cells
Cardiovascular disorders	tPA	*In vivo*	Cationic lipids	Endothelial cells
			Adenovirus	Endothelial cells
Alzheimer's disease	NGF	*Ex vivo*	Retrovirus	Fibroblasts
		In vivo	Adenovirus	Neuronal cells
Parkinson's disease	TH	*Ex vivo*	Retrovirus	Fibroblasts
			Adenovirus	Neuronal cells
AIDS	HSV-TK, HIV antigen, RevM10			T cells
				Hepatocytes
	Cytokine	*Ex vivo*	Retrovirus	Hematopoietic stem cells

Abbreviations: CFTR, Cystic fibrosis transmembrane regulator; SCID, severe combined immunodeficiency syndrome; DMD, Duchenne muscular dystrophy; ADA, adenosine deaminase; HSV-TK, herpes simplex virus thymidine kinase; NGF, nerve growth factor; TH, tyrosine hydroxylase; LDL, low density lipoprotein; tPA, tissue plasminogen activator.
*This list is not exhaustive.

of therapeutic interest such that the virus can only infect and deliver the therapeutic gene to the host but cannot replicate or cause disease. Of the various viruses that are being manipulated this way, research on retroviruses, adenoviruses and adeno-associated viruses has progressed very rapidly and deserves to be discussed in greater detail.

Viral vectors

Retroviruses as gene transfer vehicles have gained prominence because intense research on these viruses in the last two decades has generated a great deal of information on the biology and safe handling of these viruses. The work-horse for retroviral gene therapy has been the murine leukemia virus (MLV), although efforts are under way to develop HIV-based vectors so that even non-dividing cells can be infected. The first step in the development of a replication-defective recombinant retroviral vector involves the replacement of viral structural genes such as *gag*, *pol* and *env* by the therapeutic gene of interest. This vector is then transfected into a packaging cell line that provides the viral structural proteins *in trans*, so that the recombinant retroviral genome, by virtue of its packaging signal (ψ), is packaged and replication-defective retroviruses are generated. When such viruses infect the host cells, the recombinant retroviral RNA is reverse transcribed and integrated randomly into the host genome. In the absence of viral genes, the therapeutic gene is transcribed from the viral LTRs or in some cases from an internal promoter and the protein of interest is synthesized (Figure 1). Depending on the envelope protein produced in the packaging cell lines, one can generate either ecotropic viruses (which can infect rodent cells) or amphotropic viruses (capable of infecting other species including human)[5,6]. Retroviruses can infect any cell type and in order to impart specificity, the wild type envelope proteins are being modified to include proteins that can dock with specific cell surface receptors, so that they can be targeted to specific cell types. For example, an ecotropic virus which cannot infect human cells can be engineered to infect human cells expressing erythropoietin receptor, by replacing 150 amino acids of the virus envelope protein with that of erythropoietin[7]. In a different approach, the viral envelope protein was converted into an asialoglycoprotein by coupling chemically to lactose and such a virus was able to infect specifically hepatocytes which contain the asialoglycoprotein receptors. Transgene expression can also be made tissue-specific by using internal promoters that function only in specific tissues. Another improvement involves the inclusion of internal ribosome entry sites to promote cap-independent translation initiation at internal initiation codons so that multiple proteins can be expressed from a single polycistronic mRNA[6]. Several steps have been taken to prevent the generation of wild type virus such as expression of structural genes in the packaging cells under different eukaryotic promoters, mutation of the residual AUG codon in the recombinant vector, etc., so that even multiple recombination events are unlikely to generate wild type retroviral particles. The major concern of retroviral gene transfer is the insertional mutagenesis of growth regulatory genes during retroviral integration which could lead to cancer. Retroviral vectors are used mostly in *ex vivo* gene transfer experiments, although it has been shown that they can infect a regenerating liver when administered intravenously into a hepatectomized animal[8].

Adenoviruses can infect a wide range of cell types and live adenoviruses have been used as vaccines in US military personnel without any major side effects. Two serotypes, Ad5 and Ad2 have been extensively studied, the genome has been (36 kb DNA) sequenced. Of the several genes encoded by the adenoviral genome, the proteins encoded by the E1 gene are crucial for virus replication and deletion of E1 gene renders the virus replication defective. Therapeutic genes up to 3.2 kb can replace the E1 gene and introduction of the recombinant viral DNA into cells expressing the E1 gene products results in the generation of replication-defective adenoviruses[9,10]. Genes up to 7.5 kb can be accommodated by deletion of another non-essential gene,

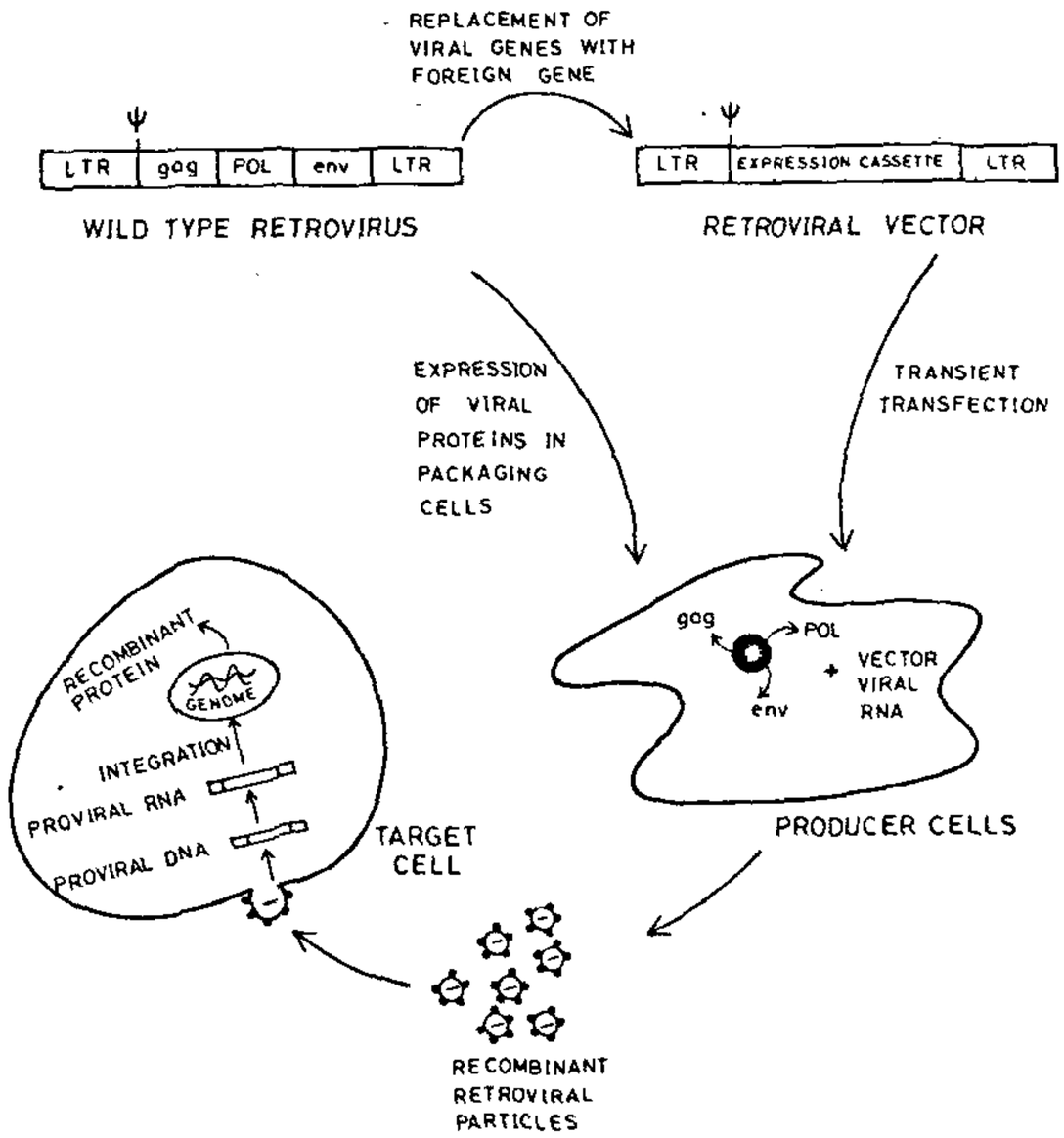

Figure 1. Retroviral gene transfer. Construction of a retroviral vector, production of recombinant retroviruses, transfection of target cells and synthesis of recombinant protein are shown (see text for details).

E3. Adenoviruses are attractive candidates for gene therapy of lung disorders because of their natural tropism for infecting respiratory epithelium[11]. Unlike retroviruses, they can infect post-mitotic cells as well and this has raised the possibility of their use for gene transfer into brain[12,13].

Adeno-associated virus (AAV), a non-pathogenic human parvovirus has aroused a lot of interest as a vector for gene therapy. AAV is a dependovirus and requires a helper virus coinfection for viral replication. In the absence of coinfection with a helper virus such as adenovirus, herpes virus or cytomegalovirus, the viral genome integrates into the human genome usually at a specific site, 19q13.3-qter. The therapeutic gene of interest is cloned between the two inverted terminal repeats or ITRs and the recombinant plasmid is transfected along with another plasmid expressing the viral structural proteins, Rep and Cap into cells infected with a helper virus. The recombinant AAV particles thus generated are purified and used for infection of target cells. The biology of AAV vectors is not as well understood as that of retroviruses or adenoviruses and this vector system is still in its infancy. The potential use of this virus as a gene transfer vector has intensified research on the life cycle of the virus and understanding the mechanism of latency, site-specific integration and development of good packaging cell lines will be the key to the success of AAV as a vector for gene therapy[2].

Non-viral approaches

Despite the fact the most of the research in gene therapy has focused on the development of viral vectors, the clinical efficacy as well as safety of these methods is still questionable. Further, the technical complexity and cost of performing viral-based gene therapy is another factor that has led to a resurgence of interest in the development of non-viral gene therapy. Unlike the viral vectors, which are used in both *in vivo* and *ex vivo* strategies, majority of the non-viral methods follow the *in vivo* approach, so that a successful non-viral technique can lead to the possibility of using genes directly as drugs. In general, non-viral methods for gene transfer can be categorized as physical and chemical methods. Of the various chemical methods, delivery of genes complexed with ligands for cell surface receptors and cationic lipids have been under intense study. The concept of delivering genes into specific tissues via receptor-mediated gene delivery was examined extensively with the liver asialoglycoprotein receptors. These receptors which are exclusively present on hepatocytes bind to certain glycoproteins lacking the terminal sialic acid. The strategy has been to couple orosomucoid (an asialoglycoprotein) to poly-L-lysine and the conjugate is condensed with plasmid DNA via electrostatic interactions. The resulting soluble complex when injected intravenously, is internalized into hepatocytes via the asialoglycoprotein receptors and transgene expression was detected up to several weeks[14] (Figure 2). This strategy was later extended to other cell surface receptors such as transferrin receptor, etc.[15]. While the receptor-mediated gene delivery is an attractive strategy as it takes advantage of the normal physiological pathway, the major problem has been the degradation of DNA by the lysosomal enzymes in the endosomes. Co-administration of lysosomotropic agents such as chloroquine has been shown to enhance gene expression. Since adenoviruses escape lysosomal degradation by acidification of endosomes, several strategies have been designed to develop adenovirus-linked molecular conjugate vectors. For example, monoclonal antibodies against a heterologous epitope on the hexon protein of the adenovirus envelope is covalently linked to polylysine which was then coupled to plasmid DNA. Such a conjugate can now interact with adenoviruses expressing the epitope on the envelope and delivery of such molecular conjugates has been shown to facilitate more efficient gene transfer than that by the conventional receptor-mediated gene delivery techniques[16]. In their present form, molecular conjugate vectors have several applications for *in vitro* use, but use in somatic gene therapy awaits more improvements in the vector design and delivery.

The observation that positively charged liposomes or cationic lipids can entrap negatively charged DNA has led to the possibility of their use as gene transfer vehicles *in vivo*. Several cationic lipid formulations are now being used for transfection of cells in culture and

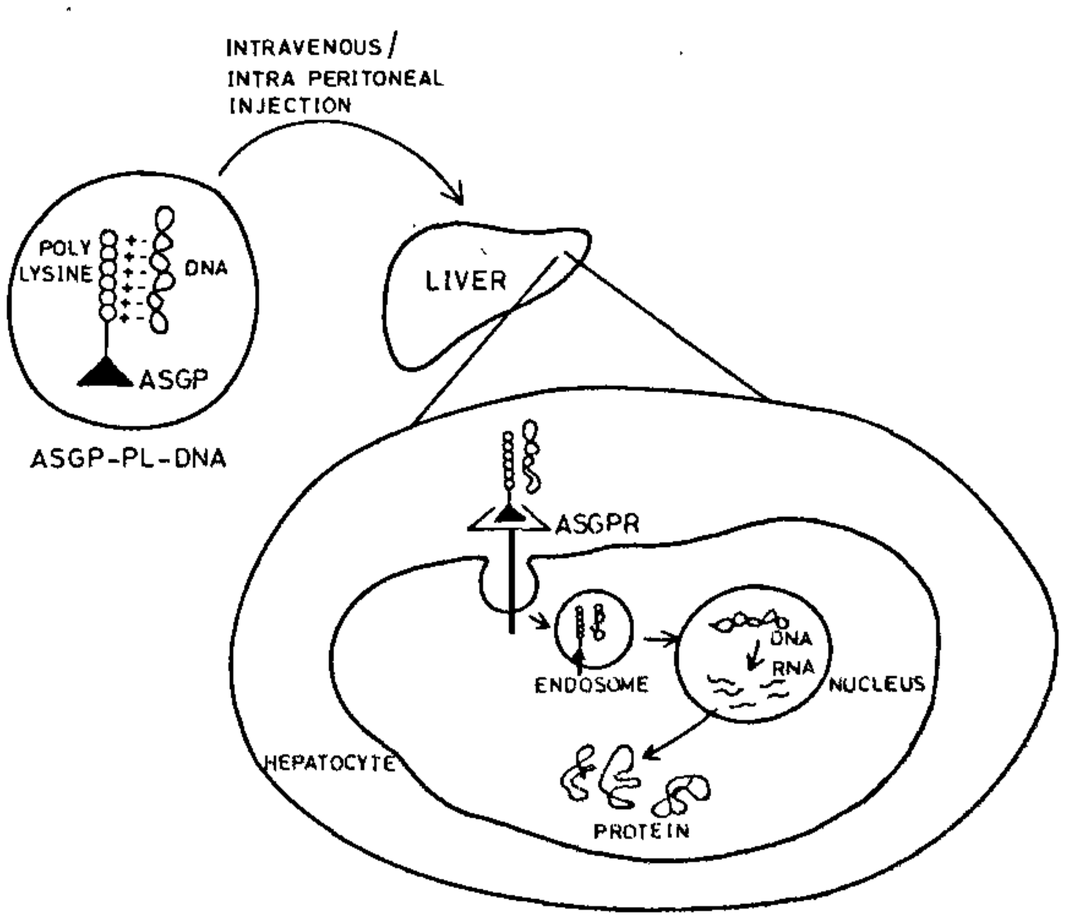

Figure 2. Receptor-mediated gene delivery. The delivery of asialoglycoprotein–poly-L-lysine–DNA complex (ASGP–PL–DNA) into hepatocytes, its internalization into hepatocytes via the asialoglycoprotein receptor (ASGPR), delivery of DNA into the nucleus and synthesis of recombinant protein are depicted.

some of these have been shown to promote gene delivery into a variety of organs *in vivo* as well[17,18]. Two potential applications of cationic lipids are the aerosol delivery of genes to lungs and in the development of cancer vaccines. The cationic lipid-mediated gene delivery to lung has advantages over adenovirus-mediated gene transfer in that, it can be administered as an aerosol and is non-immunogenic. Intratumoral injection of DNA–lipid complexes is being attempted to evoke specific immune response against a given tumour type by expression of heterologous antigens, cytokines and co-stimulatory molecules[19,20].

Amongst the physical methods of gene transfer, direct DNA injection into skeletal muscle has created a lot of excitement and has led to the possibility of using genes as vaccines. Several researchers had attempted direct gene transfer since the early 1960s, but these studies were not taken seriously due to inefficient gene transfer and low levels of expression. It was in the year 1990, Wolff *et al.*[21] reported that direct injection of plasmid DNA into skeletal muscle results in sustained expression of foreign genes but the level of expression is too low to derive a therapeutic benefit for the treatment of genetic disorders. However, researchers at Vical and Merck pursued these studies further and demonstrated that the low levels of foreign proteins synthesized in the host cells were processed along the Class I MHC pathway, leading to presentation of peptides on cell surface resulting in a potent immune response against the foreign antigens and thus was born the concept of DNA vaccine or genetic immunization. These researchers demonstrated that immunization by direct injection of plasmid DNA encoding influenza nucleoprotein protects mice from a lethal challenge of influenza virus[22]. In the last few years, these results were confirmed with a variety of genes encoding several viral and parasite antigens and many of these studies have now been approved for clinical trials in the United States. Direct injection of plasmid DNA encoding foreign antigens into skin has also been shown to evoke a protective immune response. Another physical method of gene transfer that is becoming popular is the delivery of DNA using 'gene gun'. This method, developed originally for transformation of plants, involves delivery of genes by bombardment with gene-coated gold particles into tissues such as liver, skin, mammary gland and muscle. Direct gene delivery is now being used to map promoter elements that regulate gene expression *in vivo*, in transfection of herpes simplex thymidine kinase genes into melanomas to render them sensitive to gancyclovir and as a vaccination strategy[23–26]. Thus, direct gene transfer is one example of a technique that failed to make an impression in the treatment of genetic disorders but made an enormous impact in the field of vaccine development and is being heralded as the third vaccine revolution. Other physical methods such as electroporation and microinjection have only limited scope in somatic cell gene therapy and their use is largely restricted to transfection of cells in culture and transgenic animal research.

The success of gene therapy depends not only on the gene delivery mechanism but also on the choice of target tissues. Cell types which can grow and divide *in vitro*, such as keratinocytes, myoblasts, hepatocytes, endothelial cells and hematopoietic stem cells, are amenable for both *ex vivo* and *in vivo* gene delivery strategies whereas *in vivo* methods are preferred for cell types such as neuronal cells which are not easily amenable for *ex vivo* manipulations. The choice of tissue also depends on the function of the gene product. In case of diseases such as hemophilia, the gene can be delivered and expressed in any tissue, provided that the protein is released into the blood stream. In case of diseases such as cystic fibrosis and Duhchenne's muscular dystrophy, the gene should be delivered to the specific cell types where the gene function is deficient, in order to derive the therapeutic benefit. Further, the ease and efficiency with which the transfected cells can repopulate the affected tissue is another criterion that poses limitations on *ex vivo* gene therapy. One of the main targets for *ex vivo* gene therapy is the hematopoietic stem cells and retroviral gene transfer has been the most preferred method of gene transfer into these cells. In case of gene therapy for neurological disorders, gene transfer is being attempted using adenoviral and herpes viral vectors. While *ex vivo* gene transfer is usually performed with autologous cells, heterologous cells or even immortalized cells placed in immuno-isolation devices have been used, although the potential safety concerns make them less attractive for use in humans[27]. Antisense gene transfer is another attractive strategy for the treatment of disorders such as β-thalassemia, in which accumulation of α-globin chains in red blood cells results in their premature destruction. For example, infection of K562 erythro-leukemia cells with AAV expressing human α-globin gene in antisense orientation results in significant inhibition of the endogenous α-globin gene[28].

The potential of a gene delivery system is first ascertained in cultured cells or in animal models, using reporter genes such as luciferase, growth hormone, β-galactosidase, etc. Based on these studies, pre-clinical trials are conducted in animal models to evaluate the levels and duration of expression of specific therapeutic genes. The clinical efficacy of the gene therapy procedure is then evaluated in human patients. Prior permission from specific regulatory agencies is necessary for performing gene therapy in humans. The Recombinant DNA Advisory Committee (RAC) and the Food and Drug Administration (FDA) in the United States and the Gene Therapy Advisory Committee (GTAC) in the United

Kingdom are empowered to authorize the conduct of such clinical trials in these countries. Several other countries such as The Netherlands, Italy, Germany, France, China, Japan, Switzerland, Sweden and Austria have also evolved guidelines for gene therapy research and initiated clinical trials for human gene transfer. These guidelines are being formulated in India as well.

Problems and prospects

The viral and non-viral gene delivery strategies have been fairly successful in cell culture systems and animal models but the therapeutic success of clinical trials in humans still remains questionable. Two types of clinical trials are being conducted in humans using these vectors: gene marking and therapeutic studies. In gene marking experiments, human cells which have a potential therapeutic use, such as hematopoietic stem cells, T cells, tumour-infiltrating lymphocytes, malignant cells, etc., are removed from the patient, cultured and transfected with retroviral vectors encoding marker genes and re-introduced into the patient from whom the cells are removed. At different periods after re-introduction, cells are recovered and the presence of marker gene or its products is examined. Such studies have demonstrated that gene transfer is feasible in humans and depending on the vector system used, marker gene expression could be detected for a period ranging from a few days up to three years[29-31]. Cell-marking studies have also been used to detect residual cancer cells in the marrow infused into patients during autologous bone marrow transplantation, for treatment of diseases such as leukemia[29]. Thus, cell-marking studies have been very useful in demonstrating the feasibility and safety of human gene transfer, especially using the retroviral vectors. On the contrary, the therapeutic trials are aimed at transfer of therapeutic genes into patients for treatment of specific genetic disorders and cancer. Several therapeutic clinical trails have been initiated in the last few years and the results of some of these are now available. Some examples of successful *ex vivo* human gene transfer studies are: transfer of adenosine deaminase (ADA) gene transfected T cells or cord blood cells to children suffering from adenosine deaminase deficiency leading to severe combined immunodeficiency (SCID)[32-34], transfer of autologous cells encoding cytokine genes into cancer patients, transfer of autologous hepatocytes encoding low-density lipoprotein (LDL) receptor for individuals with familial hypercholesterolaemia[35], intracerebral transfer of fibroblasts expressing herpes simplex virus thymidine kinase gene for the ablation of brain tumours[31], etc. Examples for successful *in vivo* gene transfer are: delivery and expression of the gene encoding cystic fibrosis transmembrane conductance regulator (CFTR) in the pulmonary epithelium of cystic fibrosis patients using adenoviral vectors and cationic lipids[36,37]; human leukocyte antigen (HLA-B7) and β2-microglobulin gene transfer in tumours by cationic lipid-mediated gene transfer[38,39], etc. In many of these clinical trials, patients continued to receive conventional drug and/or protein replacement therapies along with gene therapy leading to difficulty in the interpretation of results. These problems are discussed below with specific examples:

In the year 1990, a clinical trial was initiated in two children suffering from ADA deficiency, using an *ex vivo* retroviral gene transfer protocol involving autologous T cells isolated from these patients[34]. Prior to gene therapy, these children were undergoing enzyme replacement therapy wherein bovine ADA conjugated with polyethylene glycol (PEG-ADA) was being administered at regular intervals for 2–4 years. In the gene therapy approach, T cells were isolated from these children, induced to proliferate *in vitro* and then transfected with a retroviral vector encoding the normal ADA cDNA. Such genetically modified lymphocytes were expanded in the laboratory and periodically infused into the autologous patients for two years and the effect of the therapy was evaluated for a period of five years. However, enzyme replacement therapy was also continued during this period and several clinical parameters such as increase in T cell numbers, ADA levels in the circulating T cells, T cell cytokine release, cytotoxic T cell activity and skin test response to common antigens were evaluated over a period of five years. Comparison of these parameters with those observed prior to the commencement of gene therapy indicated a definitive improvement, although the continued enzyme infusions during this period makes it difficult to evaluate the benefit derived from gene therapy alone. The problem with this clinical trial has been the variation in the level of ADA^+ circulating T cells seen in these two patients, which varied from 0.1 to 60%. Nevertheless, this study demonstrated that *ex vivo* retroviral gene transfer is a feasible and safe strategy for the treatment of ADA deficiency and several protocols, each using different strategies involving different retroviral vectors targeted to different cell types have been approved for clinical trials and results of these studies are still awaited[39].

Familial hypercholesterolaemia is another genetic disorder that was the target for *ex vivo* retroviral gene therapy for a long time. This genetic defect, resulting in deficiency of LDL receptors in the liver, results in high levels of serum cholesterol and LDL cholesterol, leading to premature atherosclerosis and myocardial infarction. The gene therapy protocol for the correction of this disorder involves isolation of hepatocytes from the patient by partial hepatotectomy, *in vitro* transfection with retroviral vectors carrying a normal LDL receptor gene and reintroduction of these genetically modified hepatocytes into the portal circulation of the patient.

Studies were first carried out in rabbits, later in dogs and baboons and long term expression (up to 1.5 years in baboons) of the foreign gene was demonstrated in these animals. Based on these results, a clinical trial was initiated in a 28-year-old French Canadian woman on 5 June 1992, suffering from this genetic disorder[34]. Evaluation of molecular and metabolic data over a period of 18 months after gene transfer suggested that the genetically modified hepatocytes had engrafted stably in this patient, recombinant gene expression could be detected throughout this period and there was a significant reduction in LDL cholesterol and LDL/HDL ratio. However, similar to the ADA-deficiency studies, this study was also compromised by the fact that other conventional therapies were being administered during this period. Despite this drawback, the study demonstrated that liver-directed *ex vivo* retroviral human gene therapy is a feasible and safe approach and can be applied for the treatment of other hepatic genetic disorders as well.

Of the *in vivo* approaches, introduction of adenoviral vectors encoding the normal CFTR to the nasal or bronchial epithelium of patients with cystic fibrosis has been shown to result in the expression of CFTR in the lung epithelium[36,37]. CFTR cDNA containing plasmids have been directly administered to the nasal epithelium of these patients via cationic lipid-mediated gene transfer. In all these clinical trials, evidence for transfer of normal CFTR gene into the respiratory epithelium was clearly established, however, expression is observed in only about 5% of the target cells[31]. Further, the clinical efficacy, assessed by the correction of abnormal potential difference across the nasal epithelium, is observed in only some of the patients who have undergone gene transfer. Another problem associated with adenoviral gene transfer to the lung is the local and systemic inflammatory response evoked in some patients which was not observed in animal trials[39].

In addition to the treatment of genetic disorders, the gene transfer techniques have also been applied extensively in the treatment of cancer and other acquired disorders. The cancer gene therapy strategies are of two types: correction of abnormal genotype of the tumour cells or destruction of tumour cells. The corrective gene therapy is aimed at introducing wild type tumour suppressor genes or genes encoding dominant negative mutants of oncogenes that are mutated in the tumour cells[41]. The major limitation of this approach has been the difficulty in delivering the corrective gene into every tumour cell. Thus, efforts are largely centered on the alternative approach aimed at destruction of the abnormal tumour cells by expression of genes encoding cytokines or toxins. Autologous tumour cells are modified *ex vivo* by the transfer of genes encoding cytokines, allogenic MHC molecules or heterologous antigens to enhance their immunogenicity[42]. Another strategy has been to introduce genes encoding cytokines or tumour necrosis factor into tumour-infiltrating lymphocytes (TILs) and their autologous transfer into cancer patients[43]. In the cytotoxic gene therapy approach, genes encoding enzymes such as herpes simplex thymidine kinase, cytosine deaminase, etc., are cloned downstream of tumour-specific promoters and introduced into tumour cells. These enzymes convert pro-drugs into toxins that destroy the tumour cells. Surprisingly, even non-tranfected neighbouring cells are destroyed and this bystander effect is probably due to the transfer of the toxins between cells via cell-junctions[41,44]. Despite promising results obtained in animals, there is no convincing evidence that any of the gene therapy protocols actually led to regression of tumours in humans. Thus, the problem of inconclusive and inconsistent results obtained from clinical trials of gene therapy for genetic disorders is also true for that of cancer[45].

In the final analysis, it is clear that the move from mouse to man is not going to be easy. The conduct of clinical trails has been a great education and we now have a fair knowledge of the actual problems and pitfalls of the various gene delivery strategies. None of the clinical trials conducted so far could convincingly demonstrate the therapeutic benefit of gene therapy and has not been as effective as expected[46–48]. This is in sharp contrast to the results obtained in animal experiments which raised the expectation of gene therapy so much that the potential of gene transfer strategies became greatly exaggerated and mere conduct of a clinical trial led to a steep rise in the stock market prices of companies involved in gene therapy research. While nobody debates the importance and long term potential of human gene therapy, it is now clear that the current gene transfer techniques are largely inadequate to promote sustained, high level expression of foreign genes *in vivo*. Thus, in order for gene therapy to become a reality, it is essential that more efforts are directed towards understanding the pathophysiology of diseases as well as the mechanism of gene regulation *in vivo*. More basic research should be initiated into vector development and the role of chromatin, enhancers and locus-control regions in the regulation of gene expression *in vivo* has to be investigated in greater detail. Despite the various setbacks, gene therapy has witnessed a rapid growth in the last decade and fundamental advances made in molecular and cell biology will continue to contribute to the progress of gene therapy. For example, a recent study on hereditary tyrosinemia type I indicates that as few as 1000 transplanted hepatocytes can repopulate almost an entire liver, suggesting that retrovirally transfused hepatocytes can be induced to engraft into host liver and proliferate extensively, when appropriate selective pressures are applied[49]. Such studies could solve the problem of low efficiency of gene transfer and bring

ex vivo hepatic gene therapy much closer to reality. Gene therapy is yet to become a dream come true, but the light is seen at the end of the tunnel!

Need for gene therapy studies in India

With the explosive increase in the availability of information on human genome, several genetic disorders would become candidates for gene therapy. As already indicated, besides genetic diseases, gene therapy has the potential for treatment of several other disorders such as cancer, cardiovascular and neurological disorders and infectious diseases. At the same time, the initial hype and euphoria are giving place to a realistic assessment of the basic information that is needed to make this exciting approach a success. This 'back to the basics' approach lends tremendous scope to initiate fundamental studies on (i) design of newer vectors for gene delivery, (ii) newer approaches to systemic delivery, (iii) targeting to specific tissues and cells, (iv) stability and duration of expression of the gene introduced, (v) The status of the introduced gene *in vivo* – integrated into the chromosome or episomal?, (vi) design of appropriate animal models, (vii) assessment of risk-benefit status, and (viii) an understanding of the molecular basis of cellular humoral immune response in case of DNA vaccines.

At the present stage, only 3 or 4 laboratories have initiated research in this area in the country. At Delhi University (South Campus), delivery of macromolecules such as toxins, drugs and nucleic acids into viable cultured cells through the use of reconstituted sendai virus envelopes (RSVE or virosomes) has been demonstrated[50,51]. Since this involves fusion and delivery of genes into the cytoplasm, receptor-mediated delivery into lysosome and consequent degradation in the endosomes is circumvented. This approach needs to be extrapolated under *in vivo* conditions. At the Cancer Research Centre Bombay an approach to treat oral cancer through gene targeting to oral mucosal cells is under examination. In our laboratory, receptor-mediated targeting and expression of growth hormone gene in rat liver have been demonstrated with the use of a regulatable promoter (cytochrome P-450 promoter induced by phenobarbitone)[52]. Further, newer approaches to delivering DNA to hepatocytes by perfusion of liver with DNA-lipofectin complex have been demonstrated[53]. More recent studies have involved a comparative study of the efficiency of different carriers and routes of injection on the uptake and expression of reporter genes in experimental animals both from the perspective of gene therapy and DNA vaccination. Non-viral gene delivery strategies are being examined for their therapeutic and vaccination potential using expression vectors encoding growth hormone and antigens of rinderpest virus. There is scope to extend these genetic immunization studies to other models involving Japanese encephalitis virus, malarial parasite, etc.

The need of the hour is to initiate more studies in different systems on the various aspects mentioned earlier. The field is still at its infancy and relevant. For the first time in the history of mankind, a rational approach to actually treating a genetic disorder has become at least an experimental feasibility. A welcome fallout has been the potential of this approach to treat cancers in particular. Recognizing the importance and relevance of this approach, the Department of Biotechnology has constituted a group of experts to activate this area of research and invite proposals. It is to be hoped that the scientific community in the country would come forward to work in this area of biomedical research that lends scope for collaboration between medical and basic research scientists.

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Micelles: Self-organized surfactant assemblies

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Surfactants can self-organize under specific environmental conditions in solution to form 'micelles'. The micelles are of two types, 'normal micelles' (called micelles), which are formed by surfactant association in water or polar solvents, and 'reverse micelles', which are formed in nonpolar media. The surfactants and micellar solutions have versatile uses in the fields of chemistry, biochemistry, pharmacy, medicine and industry to augment and control solubilization, stabilization, dispersion, cleaning, rates of chemical reactions, enhanced oil recovery, etc. In this review, fundamentals of formation of micelles, their physico-chemical properties and probable uses are presented for an overall grasp of the topic of contemporary interest.

AMPHIPHILIES are chemical compounds having dual affinity for water and oil. They have distinct nonpolar (liophilic or hydrophobic) and polar (hydrophilic or liophobic) sections in their molecules. Soaps, detergents, long chain alcohols (amines, aldehydes, etc.) and lipids constitute the class of amphiphiles. They are surface active, can reduce surface tension of the medium or interfacial tension between two immiscible liquids (e.g. oil and water), can assist solubilization, cleaning, dispersion, emulsification, etc. The amphiphiles comprising soaps and detergents show a special property in solution manifesting characteristic self-organization or association called 'micelle' formation[1,2]. Under appropriate conditions, amphiphiles (mostly of lipid types) may also form 'liquid crystals' in solution. The amphiphiles that form micelles and can be potentially used for surface chemical works are termed SURFace ACTive AgeNTS or SURFACTANTS. Soaps and detergents come under this heading. Soaps are prepared by the hydrolysis (saponification) of naturally occurring fats and oils, and detergents are synthetically prepared. Big industries are busy with the production of soaps and detergents all over the world. The innumerable possibilities of such products through chemical modification and synthesis and great many uses of amphiphiles in pharmaceutical, chemical, biochemical (including biomedical) and industrial fields have added tremendous scope of research and study in this area of surface and colloid science and technology. The investigations on micelles comprise a significant share in this area, a comprehensive accounting of which is of contemporary interest. In the following sections, the overall state of the art of basic aspects of surfactants and micelles including their potential uses are presented.

Types of micelle-forming amphiphiles or surfactants

The micelle-forming amphiphiles or surfactants essentially fall in two categories, 'ionic' and 'nonionic'. They