*Review Article*

# GENE THERAPY

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Gene therapy was once considered a fantasy that would not become a reality for generations. However, it has moved from theoretical concepts to human clinical trials more rapidly than expected. A great leap in medical science has taken place on the 14th September 1990, when a group of scientists tried gene therapy for the first time. The girl suffering from severe immunodeficiency was treated by transferring the normal gene for adenosine deami-nase. It would change medicine more in the next 20 years than it did change in the past 2000 years! Thanks to the advent of DNA recombination technology, genes now can be transferred from one person to another, so that many of the genetically determine diseases are now amenable to gene therapy. It gives the world of hope where all defects could be corrected by gene replacement, and a world in which inherited diseases and cancers can be cured or at least prevented. With the improvement in the techniques, the gene therapy is likely to become a standard treatment modality in the next few decades.

There are more than 100 ongoing human gene transfer protocols, and more than 700 individuals have already participated in the human gene transfer trials (1). Gene therapy is a strategy in which genetic make up of cells are modified for therapeutic purposes. A vital gene may be missing altogether, or may be mutated to produce a wrong protein, or may express efficiently. More than 5000 monogenetic disorders exist. It is now possible to isolate, study and identify the defective gene, and replace it with normal gene. Gene therapy was initially conceptualized as a method

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to treat hereditary genetic diseases. However, the concept of gene therapy has now widened to include diseases such as AIDS, cancer and atherosclerosis.

**The Procedure** : Gene therapy involves isolation of the healthy gene along with the sequence controlling its expression; incorporation of the gene on a carrier or vector as an expression cassette; and finally the delivery of the same to the target cells. The approach for delivery may be a) ex vivo or in vitro strategy where the cells are taken from the patients and cultured in the laboratory, the modified cells are examined for their expression and administered back to the patients; or b) in vivo strategy as when the expression cassette is delivered directly to the patient.

**The Target Cells** : Gene therapy can be practiced at two different levels: a) Somatic gene therapy, which involves the insertion of a therapeutic gene into somatic cells. The technique demands the use of amenable "vectors', whose characteristics are well defined before use. b) Germ line therapy, which involves the introduction of a foreign gene into germ cells, i.e., the sperm, ovum or zygote, resulting in their expression in both somatic and germ cells produced later on. This technique is useful for treatment of inborn genetic diseases.

**The Vector** : is the most important problem. A perfect vector cell should possess the following desired qualities: it should be able to carry the full size of the genes: should be amenable to produce in large quantities : should be suitable for intravenous infusion; should be able to target specific cell types; should enter into both resting and dividing cells; should persist indefinitely either by integration into the host chromosome or by an epichromosomal mechanism; should express the gene it carriers for as long as required; and should not be recognised by the immunological system of the body. The various vector systems used for gene delivery are :

**(a) Retroviruses** : Recombinant retroviruses act as vectors for introducing new genes into a mammalian genome. They can accommodate up to 9 kb of information. Retroviruses are RNA viruses that replicate through a DNA intermediate. In practice, Moloney

Murine Leukemia Virus (MMLV) is chosen for human experiments. The gag, pol and env genes are deleted from the wild type retrovirus, rendering it incapable of replication. Then the human gene is inserted into the virus. This infectious, but replication deficient virus is introduced in a culture containing cells having gag, pol and eve genes. These packing cells provide the necessary proteins necessary to pack the virus. The packed retrovirus vector carrying the human gene now comes out of the cultured cells. These are harvested and introduced into the patient. The virus enters the target cell via specific receptor. In the cytoplasm of the human cells, the reverse transcriptase carried by the vector converts the RNA to proviral DNA, which is integrated into the target cell DNA. Along with that, the normal human gene carried by the virus is also integrated, from where the new normal gene can express. (See Figure 1). This strategy is very suitable for treatment of

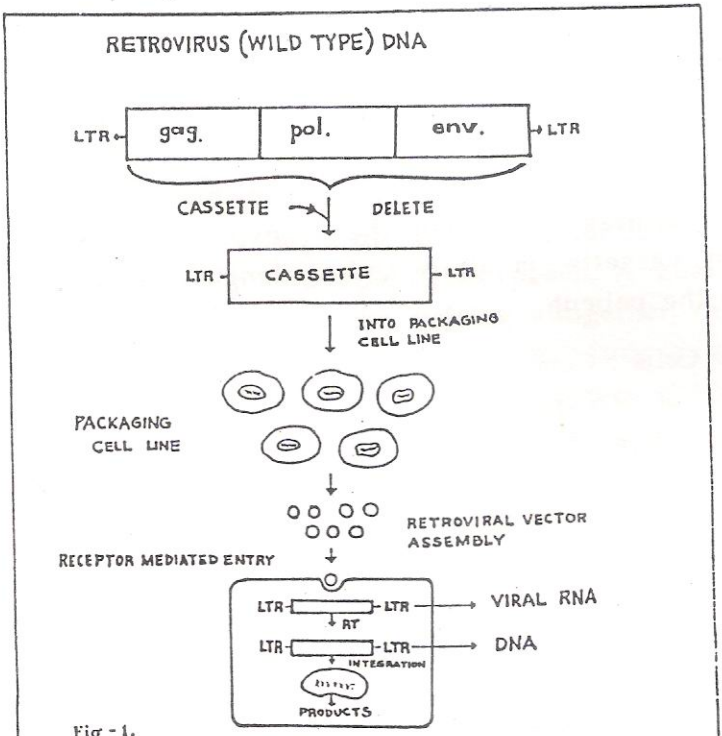


Fig - 1.

RETROVIRAL VECTOR, DESIGN AND GENE TRANSFER.

hereditary and chronic disorders. However, the vectors requires dividing cells as the targets, and allows only low titres of virus to be generated. Therefore, this mode is impractical for most in vivo strategies. Another disadvantage is the risk of toxicity associated with chronic overexpression or insertional mutagenesis. The human gene transfer was pioneered by Anderson and Rosenberg in 1990, using retrovirus vectors (2,3).

b) **Adenoviruses** : They can take up foreign genes up to 7.5 kb length. Adenoviruses are DNA viruses. The E1 gene of the virus are removed, so that they lose the ability to replicate by themselves. The human genomes are inserted and the vector is transfected into a complementary cell culture, containing the E1 sequences. The adenovirus carrying the human gene is thus replicated in the cell culture, and packed viruses are coming out. These are collected and introduced into the patient. The vector binds to the target cell through a receptor mechanism. The double stranded DNA virus with expression cassette reaches the nucleus of target cells where it is not integrated, but remains as epichromosomal (episomal) state. In contrast to retroviruses, the adenoviruses offer high titres and easier ability to infect large numbers of cells. But inside the patient, the expression is usually transient, the useful affect varying from a few week to months. Moreover, they evoke nonspecific inflammatory reaction and anti-vector cellular immunity, which limit their use in practice. The first trial with adenovirus vectors was carried by Crystal (1).

c) **Vaccinia Virus and Bacculovirus** : They are episomal in action and provide only transient expression. Not much knowledge is available about the feasibility of repeated administration of agents that provide transient expression. Hence they are not widely used.

d) **Plasmid Liposome Complex** : It is a promising nonviral vector system. Liposome are artificial lipid bilayers, which could be incorporated with plasmids carrying the normal human DNA. The complexes can enter the target cells by fusing with the plasma membrane. Most of the genetic material entering the cell is, However destroyed by the lysosomal enzymes. The small number of

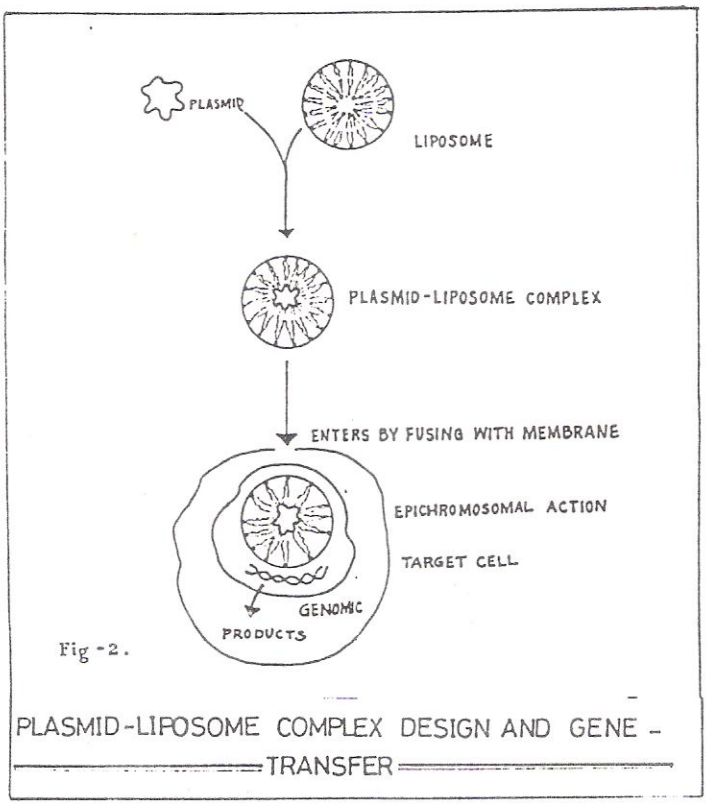


Fig -2.

PLASMID-LIPOSOME COMPLEX DESIGN AND GENE - TRANSFER

plasmids that reaching the nucleus acts as epichromosomal fashion. (See fig. 2). The advantages with this strategy is that the vector can carry expression cassettes of unlimited size, do not replicate and evoke only very weak immune responses. The design overcomes the potential safety hazards associated with viral gene sequences. However, the plasmid liposome complex lack the specificity in targeting ability. As most of the complexes are destroyed inside the h host cell, the efficiency of gene transfer is also less. The first trial with plasmid-liposome complex was carried out by Nable (4,5).

e) **Molecular Conjugate Vectors** : These synthetic molecules exploit the endogenous cellular receptor mediated pathway to transfer genes. They have the additional property of targeting specifically.

f) **Physical Methods** : The human gene DNA can be introduced by

micro injection (6) as an aerosol via liposomes; by electroporation (7) or by directly by DNA coated microparticles (gene gun) (8). But poor efficiency and short term persistence limits their use.

**ACCOMPLISHMENTS** : Although no human disease has been conclusively cured by gene therapy, promising results are obtained from ongoing clinical trials. The most dazzling results are:

**Severe Combined Immunodeficiency (SCID)** : It is caused by a deficiency of adenosine deaminase (ADA) enzyme. It is a fatal recessive disorder. The genes are located in chromosome No. 13 and 20. The normal ADA was transferred ex vivo with a retroviral vector into the T-lymphocyte precursors obtained from patients and the treated cells were re-introduced into the patients. Reports from two patients showed an increase in T cell count, and increase in DNA levels in circulating T cells. This was done in 1990 and the follow up studies showed the presence of immune function in recipients compatible with life (2,3,9).

**Familial hypercholesterolemia** : It is due to the mutation at 66th amino acid glycine being replaced by tryptophan in low density lipoprotein (LDL) receptor in hepatocytes, which renders the receptor incapable of binding LDL. This leads to atherosclerosis and heart attacks at a young age. The LDL receptor gene is located in chromosome No. 19. A retrovirus was used ex vivo to transfer normal LDL receptor cDNA to modified cells, there was a prompt decrease in LDL-cholesterol level over a period of 18 months (10).

**Cystic fibrosis** : It is common disorder affecting children, with a fatal outcome before the age of 20. The gene responsible for the disease is located in chromosome no. 7. This disorder is due to mutation in the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) gene, a gene coding for the cyclic AMP regulated chloride channel in the apical epithelium of nasopharyngeal tract. In 1990, direct administration of a vector containing CFTR DNA to the bronchial epithelium of the patient resulted in the expression of the protein product for at least 9 months (1).

**Duchenne Muscular Dystrophy (DMD)** : It is a degenerative disease of muscle with an incidence of 1 in 3500 live male births. The gene in the middle of the short arm of X-chromosome. The DMD gene produce a protein called dystrophin, containing 3700 amino acids. It is normally associated with the sarcolemma. The protein was isolated in 1987, and the gene was isolated in 1990. It is one of the largest human genes known. In 1992, the normal gene was introduced into a patients muscle cells kept in culture. The treated muscle cells were reintroduced into the patient with beneficial results.

**Hemophilia** : Gene for factor VIII was introduced into the patients fibroblasts, which were then under the skin, when they started to produce the missing protein (11).

**Lymphoproliferative disease** : occurring after bone marrow transplantation are associated with Epstein-Barr virus (EBV). EBV-specific cytotoxic T cells were infused into individuals with a retroviral vector. The preliminary results suggest that the technique could help to complications during bone marrow transplantations.

**Cancer** : human cancers are attributed to either overexpression of oncogenes or deletion of tumour suppressor genes, both due to somatic mutations (12). Hence cancer can be considered as a disease with genetic basis. The therapeutic approach could be activating a suppressor gene or turn off an activated oncogene. Gene replacement therapy with p53 (an oncosuppressor gene) gave promising results (13). Trials on genetic modification of tumour cells with a suicide gene such as the herpes simplex virus thymidine kinase gene (HSV-TK) with genecyclovir therapy are under way. The strategies for gene therapy of cancer include : (a) alteration of cancer cells to produce cytokines to alter host response to malignancy. (b) Expression of antigens on cancer cells to induce a host immune response. Plasmid liposome complex containing foreign antigen was introduced directly to melanoma, colorectal carcinoma and renal cell carcinomas with limited success (5). (c) Insertion of tumour suppressor gene sequences. (d) Introduction of drug resistance into normal cells to enable aggressive chemotherapy.

**Obstacles to Success** : The following

are the major obstacles non-encountered during the gene therapy strategies : (a) Inconsistent results. Although many studies resulted in useful life to the patient, there are many reports on failures of the protocols. (b) There are several examples in which predictions in experimental animals have been found unsafe for human systems. (c) Regarding vector production, lack of reproducibility, contamination with endotoxin and production of sufficient quantity of plasmic liposome complexes are serious obstacles encountered. (d) Lack of ideal vector. As the human applications of gene transfer are many, the ideal vector will likely be different for each applications. (e) The lack of targeting ability in non-viral vectors and the associated risks with viral vector are the real major hurdles.

**Ethical Considerations** : The social and ethical aspects were considered by the U.K. Clothier Committee, which report that somatic gene therapy is unlikely to cause any harm. However, the possibility of disruption of functional loci, insertional mutagenesis, activation of oncogenes are the possibilities in germline therapy, based on which, this strategy is not yet approved.

**Future Prospects** : The anti-sense oligonucleotide offers a powerful strategy in future. The "sense" information from the gene is interrupted by the anti-sense nucleic acid strands, produced artificially based on sequence specific hybridization. This can interfere at the DNA and mRNA levels of the gene expression, with high level of specificity (14). The human genome project, undertaken by the Nobel laureate Crick will generate around 100,000 human genes in the near future, that could be available to make expression cassettes for human gene transfer. The potential of the gene therapy technology is enormous. It is now theoretically possible to cure all the genetic diseases. However it may take several years to get these Hi-tech therapeutic modalities available for common use. Can man pay the price of associated risks and buy the innovative therapy ?

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