

GENE THERAPY

I. PRINCIPLE OF GENE THERAPY

Gene therapy is the set of approaches for transferring new genetic information into the somatic cells of a patient in order to treat his pathology. This genetic information (or gene drug) is not transmissible to the patient's progeny since it is introduced in germ cells involved in the reproduction of a species.

Several strategies are possible, depending on the pathology in question and the cells that are to be targeted by the gene-drug. There are two different methods: gene therapy *in vivo* and gene therapy *ex vivo*. In the case of gene therapy *in vivo*, the gene-drug is administered directly by injection into the diseased tissue or organ or in the bloodstream for large volume tissue targets. In the case of gene therapy *ex vivo*, the first step is the removal of target cells from the patient through surgery. That may be followed by selection and amplification if necessary. It is only during this step that the target cells are genetically modified by transfer of the gene-drug before being re-administered to the patient.

Gene therapy has already been the subject of a number of clinical trials, in particular in cancer. Rare genetic diseases are accounting for an increasingly large percentage of patients in these trials.

II. OBJECTIVES OF GENE THERAPY

The scientific and medical community is living in the hope of one day being able to treat a number of diseases by gene therapy. Here are several examples of applications, in particular in the fields of genetic or acquired diseases, cancer, infections, vaccination and enzyme deficiencies.

II.1. Gene therapy for monogenic diseases

The introduction of a gene may have different objectives depending on the origin of the disease, i.e. if it results from a loss or a gain in a given biological function.

In the case of a loss of a biological function, the aim of gene therapy is to replace the deficient gene responsible for the pathology. Using this approach, the French group of Professors Alain Fischer and Marina Cavazzana-Calvo at the Necker hospital in Paris has treated children afflicted with the X chromosome-linked severe combined immune syndrome (« bubble baby » disease). This disease is genetically characterized by a deficiency in a gene coding for a sub-

unit common to several cellular cytokine receptors. The considerable immune deficiency responsible for heightened sensitivity of infections requires that these children live in an environment protected from pathogenic microorganisms and closely monitored (in « a bubble »). In this trial conducted on several sick children, deficient cells of the bone marrow were removed, genetically modified *ex vivo* by transfer of the gene into cells in culture and then re-injected systemically. After several weeks, the immune functions of these children were reestablished, enabling them to live a normal life with a minimal risk of infections. This trial is the first success of a gene therapy.

The entire gene is not always usable if it is too large. The case of the gene coding for dystrophin is a good example and the use of a reduced form of this gene (or « minigene ») is being investigated in the context of treatment of Duchenne and Becker muscular dystrophies.

In the case of increased biological function that for example may be caused by a point mutation in a gene regulator that naturally reduces its expression, the ideal method would be the *in situ* repair of the responsible mutation by the use chimeric DNA/RNA oligonucleotides: this is called chimeraplasty. By hybridizing with the mutated region, the chimeric oligonucleotide induces the activation of specific repair systems, composed of specific enzymes naturally present in the cell and that correct the mutation. This method is currently being assessed in pre-clinical experimentation.

II.2. Gene therapy of cancer

Several therapeutic approaches are possible, depending on the type of cancer and whether or not it is hereditary.

There are several examples of this:

- ◆ The expression of so-called « suicide » genes in the tumor that can transform a precursor into a cytotoxic drug.
- ◆ The expression of genes that can be recognized by the immune system could cause the death of tumor cells that are considered as foreign to the organism.
- ◆ The transgene provided could produce proteins in cancer cells *in situ* that favor the uptake of a systemically administered toxic substance.
- ◆ The transgene provided may produce an angiogenesis inhibitor, i.e. a substance that blocks the formation of blood vessels that irrigate the tumor. The disappearance of the tumor would thus result from the absence of oxygen and nutrients.

II.3. Gene therapy of infectious diseases

There are several possible strategies for the treatment of these diseases, in particular for viral diseases.

The principles could involve producing an anti-viral protein or modifying cells that are sensitive to the virus by rendering them resistant. Most ongoing research involves treatments against the HIV-1 virus responsible for AIDS and for which work has reached the clinical trial phase.

II.4. Gene therapy and vaccination

Using DNA and not the antigenic protein has several advantages for immunizing the organism. DNA is in fact an extremely stable molecule, relatively inexpensive in comparison to the corresponding protein. This is why gene therapy is undergoing rapid development in this area, all the more so since small quantities of immunogenic proteins, in this case synthesized from the transferred gene, are sufficient to induce an immunization.

II.5. Gene therapy and delivery of therapeutic proteins

In enzymatic diseases, the endogenous production of the deficient enzyme by gene therapy may replace the daily administration of the same purified protein. To this end, gene therapy will target an easily accessible organ, known for its important synthesis capacities. Once secreted outside the cell that produced it, the protein will be capable of remote action in the bloodstream, e.g. at another organ. The organ targeted by gene therapy could be the liver, skin or muscle. It may also be an "organoid" (implant composed of cells surrounded by a semi-permeable matrix). Hemophilia will probably be one of the first diseases to benefit from this type of therapy. A phase I clinical trial has been conducted in the United States in patients with hemophilia B in order to produce factor IX by muscle (group of C. High and M. Kay). Encouraging results have shown that this production of factor IX in muscle led to a reduction in the doses of factor IX usually taken by these patients. Other work is under way in an attempt to deliver deficient factors VIII and IX from the liver in hemophilias A and B, respectively.

This drug delivery approach is also envisioned for the treatment of other rare enzymatic diseases.

III. TOOLS USED FOR GENE TRANSFER: VECTORS

In order to provide a benefit in the treatment of a disease, a gene-drug must be able to traverse several biological barriers to first access the cell (passing through the barriers of vessels, connective tissues and the plasma membrane that surrounds the cell) and finally the nucleus by

passing through the nuclear membrane. The gene will be transcribed into a messenger RNA, in turn translated into a protein in the nucleus and under the control of promoter.

In order to ensure that all the steps are possible, the gene-drug is introduced inside a vector that may be viral or non-viral (plasmids, lipid vectors ...).

III.1. Non-viral vectors

III.1.1. Plasmids

They are of bacterial origin, but are not immunogenic, in contrast to viral vectors. There are ongoing studies to improve the efficacy of their transfer. Complementary techniques such as electroporation or co-injection with molecules favoring penetration into cells will be necessary to facilitate the transfer of this naked DNA into the cell, since the passive transfer of these molecules through cell membranes is relatively low. Nevertheless, the intensity and duration of expression of the gene remains low since the plasmid is not integrated in the genome whether or not it is self-replicating and that the number of cells having incorporated the gene is low. In comparison to other vectors, especially those of viral origin, they have the major advantage of being capable of integrating large genes, as is the example of the dystrophin gene.

An initial phase I clinical trial has been started by the company Transgène in collaboration with the Institute of Myology and the AFM in the framework of Duchenne and Becker muscular dystrophies. For the moment, it is a feasibility trial whose aim is to examine the possibility of transferring DNA complementary to the dystrophin gene (and not the entire gene with its introns, elements characteristic of eukaryotic cells and eliminated during the transcription of the gene into messenger RNA) injected directly in the muscles of patients with this disease. The goal is to determine the level of expression of dystrophin, its characteristic membrane localization, its immunological tolerance by the organism and perhaps the clinical improvement it provides if all the above points are positive. This trial includes 9 patients and should terminate around the end of the first quarter of 2002.

III.1.2. Lipid vectors

In order to facilitate the penetration of DNA in the cell by the natural phenomenon of endocytosis, the DNA can be compacted inside lipid vesicles whose structure is close to the lipid bilayer that constitutes the cell membrane. These vectors are not currently used extensively since their gene transfer efficacy is low.

III.2. Viral vectors

Viral vectors have an advantage over plasmid and lipid vectors. The wild-type viruses from which they are obtained have a natural tropism for cells whose infection is part of the indispensable cycle for viral survival.

Several types of viral vectors are already being used in clinical trials at the same time as undergoing continued optimization. The vectors are primarily retroviral, adenoviral and vectors obtained from adenovirus associated viruses (AAV) selected as a function of the cells targeted by the therapy.

III.2.1. Retroviral vectors

Retroviral vectors are currently the only ones that can be used to obtain a stable expression since they are integrated in the genome of the infected cell. The expression of transferred genes is relatively long because the transferred genes are transmitted to daughter cells at divisions. Nevertheless, their use is limited to the transfer of genes into dividing cells, since they require the rupture of the nuclear membrane to enable the transgene to penetrate the nucleus.

The use of this type of vector enabled the treatment of children with severe X chromosome-linked combined immune syndrome (« bubble babies ») by the group of Alain Fischer and Marina Cavazzana-Calvo at the Necker hospital in Paris (see above).

The use of these retroviral vectors is also limited to cells that can be easily removed for an *ex vivo* modification. They in fact are very rapidly inactivated by the complement system when they are injected systemically. Studies are currently under way to enable this systemic utilization and thus enlarge their therapeutic indications.

Other retroviral vectors could be used more broadly, since they ensure the infection of resting cells. These vectors are obtained from the family of lentiviruses. They are still under study since, among other things, their safety must be ensured.

III.2.2. Adenoviral vectors

The use of these vectors is interesting because the viruses from which they are derived have a natural tropism for the upper airways and are also capable of infecting a large number of quiescent or dividing cells. They are very frequent and are responsible for common colds (harmless infections in people not weak in terms of the immune system). They are only rarely integrated in the genome of the infected cell, thus reducing the time of expression since the transgene is not transmitted to daughter cells when the infected cell divides.

The use of these adenoviral vectors is limited by the size of the transgene to transfer (maximum of 7.5 kilo base pairs), that excludes large genes. The aim of developing new generations of adenoviral vectors is to increase the size of transgenes and also to reduce immunological risks.

III.2.3. Vectors obtained from adenovirus associated viruses (AAV)

In order to accomplish their complete replication cycle, viruses of this family require adenoviruses, explaining their name of adenovirus associated viruses. The value of their use as a vector for gene transfer is explained by the fact that they can infect a large number of cell types regardless of their state (dividing or quiescent). They are frequent in the population and cause no pathology. The size of the transgene transferred (maximum 4.5 kilo bases) is a limiting factor in the use of these vectors.