

## June 2001 - The first successful human gene therapy trial Correction of an immune deficiency

The first results of an effective gene therapy protocol have just been published by researchers from Inserm and Assistance Publique-Hôpitaux de Paris belonging to a team led by Doctor Marina Cavazzana-Calvo and Professor Alain Fischer (Unit 429 Inserm, Hôpital Necker-Enfants Malades, Paris). Two very young children with a rare severe immune deficiency (X-linked SCID) have benefited from this pioneering work. One year after the introduction of a therapeutic gene into their bone marrow cells, both children have recovered a completely normal and functional immune system. They are well and treatment-free, and now live at home. These results, published in the international journal *Science*, show for the first time that it is possible to correct a disease by means of gene therapy. The trial, sponsored by Assistance Publique-Hôpitaux de Paris, was supported by the French patient association AFM (Association Française contre les Myopathies).

The two boys, who were 8 and 11 months old when the disease was diagnosed and treated, had a rare defect of the immune system known as X-linked severe combined immune deficiency (SCID). X-linked SCID is characterized by a total lack of T lymphocytes and natural killer (NK) cells, which normally defend the body against infections. With no protection against viral or bacterial aggression, children with this disease are compelled to live isolated in sterile "bubbles" while awaiting bone marrow transplantation – previously the only effective treatment. No compatible donor could be found for the two children who underwent the gene therapy.

### A clinical gene therapy trial

This disease of the immune system is due to a genetic defect on the X chromosome. The mutation of a gene named gamma-c (c), blocks the early development of T lymphocytes and NK cells in the bone marrow and thymus. The c gene encodes part of a receptor present on the surface of NK and T cell precursors, which are found in the bone marrow. Normally the receptor should receive the signals triggered by circulating cytokines such as interleukins, transmitting the message needed to transform the precursor cells into T lymphocytes and NK cells. In children with X-linked SCID the receptor is non-existent or ineffective, resulting in the failure of the precursor cells to receive the necessary signals and therefore to develop into T lymphocytes and NK cells.

### A therapeutic gene delivered to the heart of bone marrow cells

After receiving the parents' informed consent and approval from the different commissions (see inset), the researchers conducted this gene therapy trial tempting to correct the disease. The challenge was to introduce a normal copy of the c gene into the two children's bone marrow precursor cells. It was expected that precursor cells that integrated the therapeutic gene into their genome and expressed it would have a survival advantage over genetically unmodified cells and would thus be able to reconstitute a stock of T lymphocytes and NK cells.

To transport the therapeutic gene to the heart of the cells, the researchers chose a retroviral "vehicle" that had been rendered harmless. The virus, into which the c gene was "grafted", spontaneously integrates the target cell genome. Subsequently, as the genetically modified cells divide, they transmit the therapeutic gene to their daughter cells. The vector was produced by Genopoeitic, a biotechnology company in Lyon (France) specializing in gene therapy.

The patients' cells were removed, and the gene transfer procedure was performed in the laboratory (or "ex vivo"). Briefly, the children's bone marrow was harvested from the iliac fossa, under general anesthesia, and the hematopoietic precursor cells were isolated. After 24 hours of growth in an appropriate medium, the cells were infected 3 times over 3 days by the retrovirus containing the therapeutic gene. The treated cells were then re-injected intravenously to the two children.

### The disease corrected

#### A reconstituted stock of lymphocytes

The first T lymphocytes were detected in the children's blood between 1 and 2 months after the treatment. T cell production continued to increase, reaching normal levels – comparable to those in healthy children of the same age – after 4 to 6 months. NK cells emerged gradually, reaching normal levels after 5 months in both children. Now, one year after treatment, the levels of T lymphocytes and NK cells remain normal and stable in both children.

Most importantly, these newly appeared lymphocytes can react against bacteria and viruses. For example, the children have developed a normal immune response after vaccination against diphtheria and tetanus toxoid and poliovirus.

### A very rapid clinical improvement

The first signs of a clinical improvement showed very rapidly, and both children were able to leave their sterile confinement three months after being treated. They were able to go home, with no need for further treatment. Their growth and psychomotor development are entirely normal. No side effects were observed.

### Still some unknowns

Will the benefit of this therapy be temporary or permanent? It is not yet possible to know, for two reasons. Bone marrow contains T lymphocyte and NK cell precursors at various stages of maturation, and it is not yet possible to know which type of precursor integrated the therapeutic gene. Ideally, if the gene was integrated into the most primitive precursor cells, i.e. bone marrow stem cells, the effects of treatment might persist indefinitely, as these cells have the capacity to divide infinitely and therefore to transmit the gene to all daughter T cells. In contrast – and this seems more likely – the gene may have been integrated by precursors at a more advanced stage of maturation. This would mean that the number of lymphocytes they can generate will be limited. Nevertheless, T lymphocytes have a long life-span, and the beneficial action of the gene should be felt for several years (5 to 10 years, perhaps more).

The second unknown is whether the therapeutic gene will eventually stop being expressed. Indeed, previous work has shown that cells can stop expressing grafted genes, although why and when this occurs is not known.

According to the researchers, if the clinical benefit of this therapy one day disappeared it would always be possible to renew the gene transfer process or to perform bone marrow transplantation.

### Future perspectives

Despite the uncertainty over how long the therapeutic gene will "work", these results show, for the first time, that a disease can be corrected by means of gene therapy.

Three other children with SCID have since been treated in the same way by Marina Cavazzana-Calvo and Alain Fischer's team. For two of them, the clinical benefit is identical to that seen in the first two children, although follow-up is shorter (6 and 3 months). For the fifth child (6 months follow-up) the treatment doesn't seem efficient, probably because of its critical clinical state when the therapy started. All five children are monitored very closely by the scientific and medical teams.

In the future, other forms of hereditary immune deficiencies could be treated in this way, although the precise protocol used here may not be suitable: new strategies adapted to each disease and each affected tissue will have to be investigated and developed.

For further information

Sources

" Gene therapy of human severe combined immunodeficiency (SCID)-X1 disease "

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Science, 2000, vol 288, N°5465, pp 669-672

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A clinical trial done in optimal safety conditions

The clinical gene therapy trial conducted by Marina Cavazzana-Calvo and Alain Fischer's team was carried out only once a number of experimental studies had guaranteed the feasibility of this type of approach.

The work started in 1993. In 1996 it was shown that a gene could be efficiently introduced into lymphocyte precursors taken from the bone marrow of patients with X-linked SCID. Tests subsequently done on mice with the same immune deficiency showed, in 1998, that the disease could be corrected with no particular toxicity.

Strictly regulated

This gene therapy trial, sponsored by Assistance Publique-Hôpitaux de Paris, received all the necessary authorizations set out in two laws: a 1993 law related to the use and dissemination of genetically modified organisms, and a patient-protection law (Loi Huriet) passed in 1988.

Marina Cavazzana-Calvo and Alain Fischer's team received approval from the following bodies:

-the Genetic Engineering Commission, overseen by the Ministry of Education, Research and Technology, defined the level of confinement required for experimental protocols;

-the Biomolecular Engineering Commission, overseen by the Ministries of agriculture and of the environment, was responsible for assessing potential risks for the environment;

-the Viral Safety Commission, overseen by the French agency for health product safety (AFSSAPS), examined the products used in experimental protocols and assesses the risk of viral infection;

-the AFSSAPS Gene Therapy Commission, examined the design of experimental protocols.

Once these authorizations had been obtained the protocol was submitted to the Hôpital Cochin Advisory Board on the Protection of Persons Participating in Biomedical Research (Ethics Committee), which examines the validity of biomedical research, focusing on patient safety and information (Huriet's law).

The laboratory of genetic and cellular therapy

The bone marrow cells into which the therapeutic gene was introduced were treated in the laboratory of genetic and cellular therapy at Hôpital Necker-Enfants Malades. This laboratory, inaugurated in 1996, meets the quality and safety criteria required for ex vivo gene therapy trials, one of which is a confined atmosphere.

The laboratory is designed to avoid dissemination of genetically modified vectors in the environment, to guarantee that the patient is treated with totally sterile cells, and to protect the laboratory personnel. The entry is controlled, and rules of good practice are scrupulously respected.

The following partners were involved in the creation of this laboratory, which was founded by Assistance Publique-Hôpitaux de Paris, INSERM and the Pasteur Institute: AFM, Fondation de France, the association Vaincre les Maladies Lysosomales, the Association de Lutte contre les Déficiés Immunitaires Héritaires, the State departments of research, health and social security, and Région Ile-de-France.

For further information

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