Reviews

Human gene therapy: possibilities and limitations

by Hj. Müller

Dept. of Human Genetics, University Children's Hospital, CH-4005 Basel, and Laboratory of Human Genetics, Dept. of Research of the University Clinics, Kantonsspital, CH-4031 Basel (Switzerland)

Summary. Gene therapy provides new options for medicine, and implies new responsibilities for making decisions relating to the limitations which reason and morality impose upon manipulation of the human genome for the benefit both of patients and of society. This contribution outlines the possibilities and limits of gene therapy in man from a medico-technical viewpoint.
Key words. Human gene therapy; medical and ethical issues; hereditary diseases; gene transfer systems; in vitro fertilization; embryo transfer.

Ethical and legal issues concerning the 'new genetics' have become popular topics for general discussion. However, the rapid development of medical and biological research contrasts with a steadily growing information deficit among the public. For this reason physicians and scientists are called upon to inform political, religious and other circles in such a way as to provide a factual basis for the discussion relating to the limitations which reason and morality impose upon manipulations of the human genome. Only then will the conditions for a meaningful bioethical evaluation of the existing problems be fulfilled.

Recent progress in molecular genetics offers the possibility of inserting human genes into human cells. This could assist in the treatment of a few life-threatening or severely disabling hereditary diseases and possibly of some other conditions[10]. Gene therapy would then be of real assistance to sick people, if no other forms of treatment were available. On the other hand the measures employed in gene therapy should be safe and not result in new problems which might impair the physical and mental health of the treated patients or their environment. In this review, methodological aspects of possible human gene therapy are considered in the light of the possible practical applications of this new form of medical treatment.

In the discussion on the possible applications of gene therapy, a distinction must be made with regard to the following two aspects: 1) the molecular manner by which the genome of the patient's cells is modified, and 2) whether the treatment is to be carried out on germ-cells or embryonic cells, or on somatic cells. Genetic changes in somatic cells are not transmitted to the progeny of the patient.

Methods of gene therapy

The following forms of gene therapy can be envisaged:
1) the introduction of a gene at an undefined place in the genome, when a functional genetic factor is missing (gene insertion),
2) selective replacement of an abnormal gene with an own or a foreign normal gene (gene-substitution),
3) selective reverse mutation of an abnormal gene into its original state (e.g. polynucleotide-directed mutagenesis) or destruction of the abnormal gene (gene modification),
4) influencing the regulation of a specific gene.

With the currently available methods of genetic engineering it is possible to isolate a given human gene, to analyze it and to insert it, although unselectively, into the genome of another human cell (gene addition). However, at present, human genetic elements cannot be either individually exchanged, manipulated or individually controlled in their function.

Techniques of gene insertion

The DNA that contains the normal gene can be administered to human cells in several ways; the following techniques are used for gene insertion:
1) injection of the gene: the gene is injected with a very fine needle into the cell being treated. This method has the drawback that only a small number of cells can be treated in each experiment. In mice, several genes already have been micro-injected into the pronuclei of fertilized mouse eggs (see 5). The gene is integrated in about 10–25% of the fertilized ova. However, the ova very often die as a consequence of the micro-injection.
2) use of DNA viruses such as SV40 for gene transfer; however, with some viruses there is a danger that they will destroy the treated cells[11].
3) use of RNA viruses; they are probably the most suitable candidates as vectors of human genes[1, 6]. In some cases they have performed the function spontaneously by integrating e.g. oncogenes into their own genome. In addition, they can be genetically modified in such a way as to fulfill only the function of gene transfer[6].
4) calcium phosphate treatment[15]; this is an old and inexpensive, but not very efficient method. Calcium phosphate precipitation is induced by the addition of DNA/$CaCl_2$ solution into an isotonic, buffered phosphate solution. The cells to which the gene is to be transferred are then treated with the precipitate which is formed after approximately 30 min. However, the yields of gene transfer are small, particularly in the case of relatively large molecules.

5) electroporation[12]; in this method a brief electric impulse with a given field strength is used to increase the permeability of a membrane in such a way that DNA molecules can also penetrate into the cells.
6) membrane fusion[17], as for instance with liposomes containing the DNA which is to be transferred. The DNA is conveyed into the recipient cells by the fusion.

None of these techniques allows the transfer of a defined number of genes into any given cell, a fact which severely limits the application of gene therapy.

Gene therapy of human germ and embryonic cells

For purely scientific and medico-technical reasons, direct interventions into the genome of human gametes (spermatozoa and ova) or their precursors, and into that of zygotes and early embryos (pre-embryos), are outside the realms of possibility and will remain so in the near future. Experiments in the field of basic research into developmental biology, in which foreign genes are transferred to fruit flies[19] or laboratory mice[5,13] in early stages of development do not aim so much at creating a basis for gene therapy as at providing an insight into the genetic control of the complex embryonic development and the manner in which genes function. The 'transgenic mice' in which a foreign gene is usually transferred to one pronucleus of the fertilized ovum are among the current models of basic research into developmental biology and genetics.
The medical-technical impediments to gene therapy in human gametes and embryos are as follows:
1) even in the presence of a high genetic risk in one parent, only a portion of his/her gametes are carriers of the abnormal gene. However, treatment would be justifiable only for gametes and embryos which are genetically abnormal. It is not possible to detect a single abnormal gene in a single cell or in a very small number of cells. Furthermore the cells must be destroyed for DNA extraction, which is a prerequisite for DNA diagnosis. In the case of the embryo it may be possible to find a way out by separating a small number of cells from it, multiplying them in culture and conversing the rest of the embryo until it has been ascertained whether an attempt at therapy is indicated or not.
2) before returning the treated embryo to the uterus it would be necessary to make certain that the transferred gene had integrated itself into the genome in such a way that it is able to perform its function and that it has no undesirable effects on other genes located in its vicinity. It is often not possible to demonstrate a 'clinical cure' of a diseased embryo. The correct regulation of a gene for the alpha- or beta-chain of hemoglobin cannot be determined in early embryonic stages since it is physiologically inactive in these phases. Nor is it possible to predict the consequences of the inserted gene for the genetic factors located in its vicinity. There is also the risk of disrupting the integrity of resident cellular genes by insertional mutagenesis. The available data suggest that 10%–20% of transgenic mice may harbor recessive mutations of essential genes[13]. However, a fetus which had not been successfully treated or whose health had been impaired by the treatment could occasionally be identified by prenatal diagnostic procedures.
3) an important stage in a successful treatment involving a human embryo would require embryo transfer (ET). This technique of reproductive medicine is also associated with bioethical problems[9] and has limited success. The pregnancy rate per attempt of ET following in vitro-fertilization is between 10 and 15%[16], of which slightly more than half of the pregnancies end in a birth. It has to be considered that these results are often achieved by transferring several embryos together. This situation would severely limit the chances of gene therapy in human embryos.
4) New methods of medical treatment would first be tried in a small group of patients who have no prospect of other medical aid. The initiation of such a therapeutic experiment in human embryos presents special problems; what happens if as a result of the treatment a sick or malformed child is born?

A form of treatment which achieves only occasional success, which risks killing the treated embryo, or seriously jeopardizes the health of the growing child is unacceptable. In addition, it is impossible or very difficult to assess the possible consequences for future generations of inserting genes into the germ cell line and to determine the possible long-term consequences of such interventions for the genetic characteristics of mankind. But, since the insertion of genes into fertilized animal ova is regularly carried out in basic biological research and also in animal breeding[5], it may be possible to accumulate a stock of experience which will throw another light on the use of human germ cell therapy in the future.

Gene therapy of somatic cells

For this purpose cells which do not fulfill their specific function as a result of a genetic defect are removed from the patient. It is only to these cells that a normal gene is transferred in vitro. During cell culture, it is determined whether the insertion of the gene has succeeded. The cells in which the transferred gene is functioning are multiplied and returned to the patient. Initial trials of this type of therapy are likely to be directed at alleviating a severe hereditary combined immune deficiency caused by the lack of an enzyme called adenosine deaminase[1,10]. Transfer of a good adenosine deaminase gene has now been shown to 'cure' the enzyme defect in hematopoietic cells grown in culture[20]. Nucleotide phosphorylase deficiency and HGPRT-deficiency (Lesch-Nyhan-Syndrome) are also considered as candidates for somatic gene therapy. In addition, this approach could be helpful to children whose bone marrow cells do not produce functional hemoglobin of the adult type (e.g. sickle cell anemia, thalassemias). The limitation of gene therapy of somatic cells becomes evident when one bears in mind that only very few cell types can be cultured in vitro. It is not possible to employ this treatment on brain and liver cells, in which hereditary diseases are particularly common. In addition, there is little point in treating one cell type in a genetic disease which has severe effects on different organs. Thus of the more than 3000 monogenetically transmitted diseases there remains only a small group in which

somatic gene therapy is likely to be of practical use in the near future[10].

Gene therapy of somatic cells differs little in principle from the generally accepted organ on cell transplantation. The transferred gene ceases to exist with the death of the individual whose cells have been treated. The issues raised by somatic gene therapy are similar to those relating to any new type of medical treatment which may involve certain risks.

Technical prerequisites for the gene therapy of somatic cells

Much remains to be learned about how to introduce a defined number of genes into foreign cells and how to achieve their proper regulation in the new environment before the first experiments can be performed in man. The following requirements have to be fulfilled:

1) For gene therapy it is necessary for the defect to be known at the DNA level and for the normal gene to have been isolated and cloned. This requirement is fulfilled today in only a small fraction of monogenic diseases[2].

2) It must be possible to collect an adequate number of suitable target cells. Many differentiating cell systems are organized in a hierarchy in which large populations of mature differentiated cells arise from a very small population of stem cells. It is estimated that there is only one stem cell for every 1000 or 100,000 bone marrow cells. If the gene is not transferred to such a stem cell but into a cell or cells which are already on the way to final differentiation, even a successfully treated cell line will die out again. It is also necessary to insert a functioning gene into a sufficient number of stem cells, especially since the expression of the gene product in the transfected cells is often less than that in normal ones. Cline et al.[4] implanted selective markers along with the transferred genes because in transfection experiments performed in animals these proved to be useful for stabilizing the gene that was linked with them. Thus, for example a methotrexate-resistant dihydrofolate reductase gene was linked with the beta chain gene. It was also hoped that bone marrow cells with the recombinant DNA could be placed at an advantage in the patient suffering from thalassemia, by giving the patient a high dosage of methotrexate.

3) Finally, it would also be appropriate, before effecting gene therapy in man, to know what are the regulatory elements which selectively control the activity of a specific gene in a specific cell type. This important prerequisite for gene therapy in man has not yet been fulfilled. It seems that eukaryotic cells can inactivate genes regulated by control sequences of the viral vector.

4) The requirements for an in vivo somatic therapy by introducing genes systematically, e.g. by vaccination with the vector are not fulfilled.

Gene therapy for non-hereditary diseases

The potential range of applications of human gene therapy is wide. A possible use would be to implant genes into normal cells, e.g. in cells that do not originate from patients with hereditary diseases, in order to make them more resistent to cytostatic therapy or to prevent occupational diseases in individuals who are at greater risk because of exposure to certain chemicals in the workplace. However, such measures give rise to a number of severe medical and bioethical problems which need special consideration.

Concluding remarks

Hereditary diseases are often very serious conditions in which present-day medicine can do very little to help[18]. Therefore, it is understandable that new means of treating them are being sought. In this connection it must be borne in mind that there are also alternatives to gene therapy. Biotechnological methods are making available a steadily growing number of proteins for supportive treatment in which the patient is supplied with the normal gene product that he cannot himself produce. The possibilities of organ and cell transplantation have also improved. There are reports of successful bone marrow transplantation in patients with immune defects, thalassemia, osteopetrosis and Maroteaux-Lamy-syndrome[7,8,14]. Liver and kidney transplantations have also been used for the treatment of genetic diseases[3]. Another important possibility that could be envisaged would be the transfer of healthy stem cells to embryos by developmental biological manipulations in order to prevent a genetic disease from manifesting itself.

Rather than to draw attention to the hopes attendant upon gene therapy, the mass media have tended to emphasize the possibilities of its abuse. This has strengthened the impression, already held by a large section of the lay public, that scientific advances must inevitably lead to Frankenstein experiments. However, a close look at the situation shows that many of the indicated possibilities of abuse are simply not practicable. Gene therapy provides new options for medical therapy and implies new responsibilities for making decisions fairly and for the benefit both of patients and of society.

1 Anderson, W.F., Prospects of human gene therapy. Science *226* (1984) 401–409.

2 Baudet, A.L., Bibliography of cloned human DNA's. Am. J. hum. Genet. *37* (1985) 386–406.

3 Bilheimer, D.W., Goldstein, J.L., Grundy, S.C., Starzl, T.E., and Brown, M.S., Liver transplantation to provide low-density-lipoprotein receptors and lower plasma cholesterol in a child with homozygous familial hypercholesterolemia. N. Engl. J. Med. *311* (1984) 1658–1664.

4 Cline, M.J., Stang, H., Mercola, K., Morse, L., Ruprecht, R., Browne, J., and Salser, W., Gene transfer in intact animals. Nature *284* (1980) 422–425.

5 Constantini, F., and Jaenisch, R., (Eds) Genetic manipulation of the early mammalian embryo. Banbury report No. 20, Cold Spring Harbor Laboratory 1985.

6 Dick, J.E., Magli, M.C., Phillips, R.A., and Bernstein, A., Genetic manipulation of hematopoietic stem cells. Trends in Genetics *2* (1986) 165–170.

7 Donnall, Th.E., Marrow transplantation for nonmalignant disorders. N. Engl. J. Med. *312* (1985) 46–47.

8 Krivit, W., Piermont, M.E., Ayaz, K., Tsai, M., Ramsay, N.K.C., Kersey, J.H., Weisdorf, S., Sibley, R., Snover, D., McGovern, M.M., Schwartz, M.F., and Desnick, R.J., Bone marrow transplantation in the Maroteaux-Lamy-syndrome (Mucopolysaccharidosis Type VI). N. Engl. J. Med. *311* (1984) 1606–1611.

9 Müller, Hj., Human in vitro fertilization and embryo transfer: expectations and concerns. Experientia *41* (1985) 1515–1517.

10 Müller, Hj., Gentherapie beim Menschen, eine Standortanalyse. Veröffentlichung des Schweiz. Institutes für Rechtsvergleich, Vol. 4. Lausanne 1986.

11 Mulligan, R.C., and Berg, P., Experession of a bacterial gene in mammalian cells. Science *209* (1980) 1422–1427.
12 Neumann, E., Schaefer-Ridder, M., Wang, Y., and Hofschneider, P.H., Gene transfer into mouse L-cells by electroporation in high electric fields. EMBO J. *1* (1982) 841–845.
13 Palmiter, R.D., and Brinster, R.L., Transgenic mice. Cell *41* (1985) 343–345.
14 Parkman, R., The application of bone marrow transplantation to the treatment of genetic diseases. Science *232* (1986) 1374–1378.
15 Pellicer, A., Wigler, M., Axel, R., and Silverstein, S., The transfer and stable integration of the HSV thymidine kinase gene into mouse cells. Cell *14* (1979) 133–141.
16 Proceedings of the III. World Congress of in vitro Fertilization and Embryo Transfer, Helsinki, Finland (1984).
17 Schaffner, W., Direct transfer of cloned genes from bacteria to mammalian cells. Proc. natn. Acad. Sci. USA (1980) 2163–2167.
18 Shapiro, L.J., Treatment of genetic diseases, in: Principles and Practice of Medical Genetics, pp. 1488–1496. Eds A.E.H. Emery and D. Rimoin. Churchill Livingstone, Edinburgh and London 1983.
19 Spradling, A.C., and Rubin, G.M., The effecxt of chromosomal position on the expression of the drosophila xanthine dehydrogenase gene. Cell *34* (1983) 47–57.
20 Williams, D.A., Orkin, S.H., and Mulligan, R.C., Retrovirus-mediated transfer of human adenosine deaminase sequences into cells in culture into murine hematopoietic cell vivo. Proc. natn. Acad. Sci. USA *83* (1986) 2566–2570.

0014-4754/87/040375-04$1.50 + 0.20/0

Full Papers

Changes in pulse wave velocity with age in man: a longitudinal series over 20 years

M. Monnier

(formerly: Institute of Physiology, University of Basel) Äussere Baselstrasse 91, CH-4125 Riehen (Switzerland)

Summary. Measurements of pulse wave velocity (PWV) carried out systematically in the same 60 healthy individuals over 20 years confirmed that average PWV progressively increases with age, and more rapidly after age 45. The PWV increase mainly results from the decreased extensibility of the thoracic aorta in the aortic-popliteat arterial system. This longitudinal study showed that PWVs of most individuals do not closely follow the average cross-sectional trend, but vary considerably in type. Therefore, PWV by itself is hardly a close correlate of an individual's physiological age or life expectancy. Individuals with a constantly high PWV or a late abrupt increase are exceptional; a possible relation to an increased mean arterial pressure and decreased arterial extensibility as in hypertonia is mentioned.
Key words. Pulse wave velocity; longitudinal study.

In honor of Fritz Verzár (1886–1979) who, as an all-round physiologist and a pioneer in molecular biology and experimental gerontology, devoted his life to interdisciplinary research.

This physiological investigation into aging was initiated in 1954/55 by Fritz Verzár, then Professor of Physiology at Basel University. As Verzár's successor, I continued this investigation for another 20 years, until 1976. My contribution was part of the teamwork carried out by the Basel Interdisciplinary Longitudinal Study, in association with my colleagues O. Gsell (Internal Medicine), R. Brückner (Ophthalmology) and E. Batschelet (Statistics). A first paper on age-related changes of pulse wave velocity (PWV) in man was published after the first ten years of the study[1]. The present report covers all PWV measurements over 20 years in 60 healthy male probands. During the last 10 years, our team submitted the large volume of data to statistical computer analysis (F. Gutzwiller and F. Hugenschmidt).
Previous cross-sectional studies have shown that PWV increases with age because of hardening of the arterial wall. Since PWV increases steadily with age in each individual, it might be a useful measure of physiological age. Furthermore, it might contribute to a better understanding of the physical and chemical conditions responsible for alterations of the arterial wall in physiological and pathological aging.

Methods

Measurement of PWV. We selected 60 healthy men who each contributed 5 to 6 measurements, distributed as evenly as possible over at least 15 years. Most of these volunteers, staff members of a Basel pharmaceutical company, were able to produce PWV data over a period of 20 years. Every year, measurements of PWV were taken during the same period (September/October) and at the same time of day (09.00–10.00 h) in order to avoid differences due to possible seasonal or circadian influences. Originally, PWV measurements were carried out once a year in a few probands and every second year in most of the subjects. The experiment ended in October 1976.
This long-term study was made possible by the stability of the group of probands, who remained for a long time in the same city.
PWV was measured by the method of Boucke and Brecht[2]. It is determined in the arterial circulation between the aorta (near the heart) and the foot (the arteria dorsalis pedis) in the prone position (fig. 1). On the left foot the pulse wave on the a. dors. pedis (A″ in fig. 1) was