Prospects for Human Gene Therapy in the Born and Unborn Patient

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This volume contains a wide range of articles covering exciting new areas in fetal therapy. These therapies are now in active use and many of them offer hope for even greater help for the afflicted fetus in the near future. My topic is somewhat different: gene therapy does not exist even for the born patient, much less for the fetus. But progress is rapid and the potential for alleviation of genetic disorders in the pediatric patient, and ultimately in the fetus, is very promising. In this article I summarize for the practicing obstetrician and neonatologist the current state-of-the-art of human gene therapy for the pediatric patient. Then I briefly speculate on situations in which there may be a potential advantage for carrying out gene therapy in the fetus rather than waiting until after birth.

Genetic engineering for the insertion of a gene into a human being could, in theory, be carried out in four different ways:

1. Somatic cell gene therapy: resulting in the correction of a genetic disorder in the somatic (i.e., body) cells of a patient.
2. Germ line gene therapy: resulting in the insertion of the gene into the reproductive tissue of the patient in such a way that the disorder in his or her offspring would also be corrected.
3. Enhancement genetic engineering: resulting in the insertion of a gene in a normal individual with the intention of enhancing a known characteristic; for example, the placing of an additional growth hormone gene into a normal child or fetus.
4. Eugenic genetic engineering: resulting in the attempt to alter or "improve" complex human traits, each of which is coded by a large number of genes; for example, personality, intelligence, character, formation of body organs, and so on.

Let us examine each of these four categories separately.

Somatic Cell Gene Therapy

There are many examples of genes that, when defective, produce serious or lethal disease in a patient. Gene therapy should be beneficial primarily for the replacement of a defective or missing enzyme or protein that must function inside the cell that makes it, or of a defective or missing enzyme whose absence leads to a buildup of a
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toxic level of a normal metabolite (e.g., as in the disease phenylketonuria (PKU)), or of a deficient circulating protein whose level does not need to be exactly regulated (e.g., blood clotting factor VIII, which is deficient in hemophilia). The initial candidates for gene therapy would need to satisfy several requirements. First, because this is a new unproved therapy with, therefore, uncertain risks, the initial diseases would need to be severe genetic disorders leading to severe crippling and/or early death. Thus the benefit/risk ratio would potentially be much higher than for mild disorders. Second, the disease should be correctable by treatment of the bone marrow cells, because only the bone marrow can be removed from a patient, treated ex vivo, and then returned to the patient. Third, the defect should be an enzyme with simple regulation, not a protein like hemoglobin where relatively complex regulation is involved (see following discussion). And fourth, the normal gene would need to have been cloned.

The most likely gene to be used in the first experiments attempting human gene therapy is adenosine deaminase (ADA), the absence of which results in severe combined immunodeficiency disease (in which children have a greatly weakened resistance to infection and cannot survive the usual childhood diseases). In ADA deficiency, the clinical syndrome is profoundly debilitating. The disorder is found in the patient's bone marrow, and there is no, or minimal, detectable enzyme in marrow cells from patients who have no copies of the normal gene. In these patients, the production of a small percentage of the normal enzyme level should be beneficial, and a mild overproduction of enzyme should not be harmful. In addition, the normal gene has been cloned and is available. Furthermore, severe combined immunodeficiency due to a defect in the ADA gene can be corrected by infusion of normal bone marrow cells from a histocompatible donor. Therefore, selective replication of the normal marrow cells appears to take place. This observation offers hope that defective bone marrow can be removed from a patient, the normal ADA gene inserted into a number of cells through gene therapy, and the treated marrow reimplanted into the patient where it may have a selective growth advantage. If selective growth occurs, elimination of the patient's own marrow would not be necessary. If, however, corrected marrow cells have no growth advantage over endogenous (i.e., the patient's own untreated) cells, then partial or complete marrow destruction (by either irradiation or other means) may be required in order to allow the corrected marrow cells an environment favorable for expansion. The latter situation would require much greater confidence that the gene therapy procedure would work before a clinical trial should be undertaken.

Previously, clinical investigators thought that the human genetic diseases most likely to be the initial ones successfully treated by gene therapy would be the hemoglobin abnormalities (specifically, beta-thalassemia) because these disorders are the most obvious ones carried by blood cells, and bone marrow is the easiest tissue to manipulate outside the body. Regulation of globin synthesis, however, is unusually complicated. Not only are the embryonic, fetal, and adult globin chains carefully regulated during development, but also the subunits of the hemoglobin molecule are coded by genes on two different chromosomes. To understand the regulatory signals that control such a complicated system and to develop means for obtaining controlled expression of an exogenous (i.e., inserted by gene therapy) beta-globin gene will take considerably more research effort.

What criteria should be satisfied before somatic cell gene therapy is tested in a clinical trial? Three general requirements, first presented in 1980, are that it should be
shown in animal studies that 1) the new
gene can be put into the correct target cells
and will remain there long enough to be
effective, 2) the new gene will be expressed
in the cells at an appropriate level, and 3)
the new gene will not harm the cell or, by
extension, the animal. These three requi-
sites, summarized as delivery, expression,
and safety, are examined in turn.

These criteria are very similar to those
required prior to the use of any new drug,
therapeutic procedure, or surgical opera-
tion. The requirements simply state that
the new treatment should get to the area of
disease, correct it, and do more good than
harm. The exact definitions of what is
"long enough to be effective," what is "an
appropriate level," and how much harm is
meant by "harm" are questions for on-
going discussion as more is learned about
gene therapy. Ultimately, local instituti-
unal review boards and the National Institutes
of Health (NIH), the latter through the Re-
combiant DNA Advisory Committee
(RAC) with its Working Group on Human
Gene Therapy, must decide if a given pro-
tocol is ready for human application. Once
the criteria are satisfied, that is, when the
probable benefits for the patient are ex-
pected to exceed the possible risks, then
attempts to cure human genetic disease by
treatment with somatic cell gene therapy
would be ethical. The goal of biochemical
research is, and has always been, to allevi-
ate human suffering. Gene therapy is a
proper and logical part of that effort.

Delivery

At present, the only human tissue that can
be used effectively for gene transfer is
bone marrow. No other cells can currently
be extracted from the body, grown in cul-
ture to allow insertion of exogenous genes,
and then successfully reimplanted into the
patient from whom the tissue was taken. In
the future, as more is learned about how to
package the DNA and to make it tissue spe-
cific, the intravenous route would be the
simplest and most desirable. However,
attending to give a foreign gene by injec-
tion directly into the bloodstream is not ad-
visable with our current state of knowledge
since the procedure would be enormously
inefficient and there would be little control
over the DNA's fate.

Bone marrow consists of a heterogene-
ous population of cells, most of which are
committed to differentiate into red blood
cells, white blood cells, platelets, and so on.
Only a small proportion (0.1–0.5%) of nu-
cleated bone marrow cells are stem cells
(that is, blood-forming cells that have not
yet differentiated into specific cell types
and that divide as needed to maintain the
marrow population). In gene therapy, it
would be these rare, unrecognizable stem
cells that would be the primary target. Con-
sequently, a delivery system useful for gene
therapy must be efficient.

Several techniques for transferring
cloned genes into cells have been devel-
oped. Each procedure is valuable for
certain types of experiments, but none can
yet be used to insert a gene into a specific
chromosomal site in a target cell. At
present the most promising approach for
gene transfer into humans employs
trolovirus-based vectors carrying exoge-
nous genes.

Vectors derived from retroviruses pos-
session several advantages as a gene delivery
system. First, up to 100% of cells can be
infected and can express the integrated vi-
ral (and exogenous) genes. Second, as
many cells as desired can be infected simulta-
necessarily; 10⁶ to 10⁷ is a convenient num-
ber for a simple protocol. Third, under
appropriate conditions, the DNA can inte-
grate as a single copy at a single, albeit
random, site. Finally, the infection and
long-term harboring of a retroviral vector
usually do not harm cells. Several retroviral
vector systems have been developed; those
projected for human use at the present
time are constructed from the Moloney
murine (mouse) leukemia virus. Evidence
obtained from studies with experimental animals and in tissue culture indicates that retroviruses can be used as a reasonably efficient delivery system.\textsuperscript{5-9}

An ideal delivery system would be tissue specific. When a genetic disorder is in the blood cells, the isolated bone marrow can be treated. But no other tissue can be removed, treated, and replaced at present. Since many viruses are known to infect only specific tissues (that is, to bind to receptors that are present only on certain cell types), a retroviral particle containing a coat that recognizes only human blood-forming cells would permit the retroviral vector to be given intravenously with little danger that cells other than those in the marrow would be infected. In the future, such specificity could permit the liver and brain, for example, to be treated individually. In addition, the danger of inadvertently infecting germ cells could be eliminated. One problem however, is that cell replication appears to be necessary for retrovirus integration. It would not be possible to infect nondividing brain cells, for example, as far as we now know.

The optimal system not only would deliver the vector specifically into the cell type of choice but would also direct the vector to a predetermined chromosomal site. Specific insertion into a selected site on a chromosome can be achieved in lower organisms but has not yet been possible in mammals.

**Expression**

In order for gene therapy to be successful, there must be appropriate expression of the new gene in the target cells. Even when a delivery system can transport an exogenous gene into the DNA of the correct cells of an organism, it has been a major problem to get the integrated DNA to function. A vast array of cloned genes have been introduced into a wide range of cells by several gene transfer techniques, but "normal" expression of exogenous genes is the exception rather than the rule.\textsuperscript{4}

Expression of exogenous genes carried by retroviral vectors into intact animals via treated bone marrow cells has now been reported by a number of laboratories. Most studies have demonstrated the expression of an antibiotic resistance gene in mice,\textsuperscript{6,7,9} but one has reported production of a human enzyme (HPRT) in the blood-forming tissues of mice.\textsuperscript{10} Our laboratory has also demonstrated low levels of expression of the human ADA gene in the bone marrow cells of lethally irradiated non-human primates that were reinfused with their own bone marrow cells after the cells and been treated in vitro with an ADA retroviral vector.\textsuperscript{11} These several reports provide hope that vectors can be built with all the regulatory signals necessary to produce correctly controlled expression of exogenous genes in target cells in vivo.

**Safety**

Finally, a human gene therapy protocol must be safe. Although retroviruses have many advantages for gene transfer, they also have disadvantages. One problem is that they can rearrange their own structure, as well as exchange sequences with other retroviruses. In the future it might be possible to modify noninfectious retroviral vectors in such a way that they remain stable. At present, however, there is the possibility that a retroviral vector might recombine with an endogenous viral sequence to produce an infectious recombinant virus. Properties that such a recombinant would have are unknown, but there is a potential homology between retroviral vectors and human T-cell leukemia viruses so that the formation of a recombinant that could produce a malignancy is a possibility. There is, however, a built-in safety feature with the mouse retroviral vectors now in use. These murine structures have a very different sequence
from known human retroviruses, and there appears to be little or no homology between the two. Therefore, it should be possible, with continuing research, to build a safe retroviral vector.

**Conclusion**

It now appears that effective delivery—expression systems are becoming available that will allow reasonable attempts at somatic cell gene therapy in the pediatric patient. The first clinical trials will probably be carried out within the next year. The initial protocols will be based on treatment of bone marrow cells with retroviral vectors carrying a normal gene. The efficiency and safety of the procedures are the remaining issues. Patients severely debilitated by having no normal copies of the gene that produces the enzyme ADA are the most likely first candidates for gene therapy.

It is unrealistic to expect a complete cure from the initial attempts at gene therapy. Many patients who suffer from severe genetic diseases, as well as their families, are eager to participate in early clinical trials even if the likelihood is low that the original experiments will alleviate symptoms. However, for the protection of the patients (particularly since those with the most severe diseases and, therefore, the most ethically justifiable first candidates are children), gene therapy trials should not be attempted until there are good animal data to suggest that some amelioration of the biochemical defect is likely. Then it would be necessary to weigh the potential risks to the patient, including the possibility of producing a pathologic virus or a malignancy, against the anticipated benefits to be gained from the functional gene. This risk-to-benefit determination, a standard procedure for all clinical research protocols, would need to be carried out for each patient.

In summary, institutional review boards and the NIH should carefully evaluate therapeutic protocols to ensure that the delivery system is effective, that sufficient expression can be obtained in bone marrow cultures and in laboratory animals to predict probable benefit, even if small, for the patient, and that safety protocols have demonstrated that the probability is low for the production of either a malignant cell or a harmful infectious retrovirus. Once these criteria are met, I maintain that it would be unethical to delay human trials. Patients with serious genetic diseases have little other hope at present for alleviation of their medical problems. Arguments that genetic engineering might someday be misused do not justify the needless perpetuation of human suffering that would result from an unnecessary delay in the clinical application of this potentially powerful therapeutic procedure.

**Germ Line Gene Therapy**

The second way that genetic engineering could be used to insert genes into humans, gene therapy of germ line cells, would require a major advance in our current state of knowledge. It would require that we learn not only how to insert a gene into the appropriate cells of the patient's body but also how to introduce it into the germ line of the patient in such a way that it would be transmitted to offspring and would be functional in the correct way in the correct cells in the offspring. With the small amount of information now available from animal studies, the step from correction of a disorder in somatic cells to correction of the germ line would be difficult.

Germ line transmission and expression of inserted genes in mice have been obtained by several laboratories but with a technique that is not acceptable for use in human patients, namely, the physical microinjection of fertilized eggs. Microinjection into tissue culture cells has been used for a number of years and has the advantage of high efficiency (up to one tissue culture cell in five injected can be per-
manently transfected). However, the distinct disadvantage is that only one cell at a time can be injected. Transfection of a large number (like $10^6$) of blood-forming stem cells is not feasible.

Microinjection has been used with considerable success in transferring genes into mouse zygotes. DNA can be microinjected into one of the two pronuclei of a recently fertilized mouse egg. This egg can then be placed into the oviduct of a pseudopregnant female, where it can develop into a normal mouse carrying the exogenous DNA in every cell of its body, including its germ cells. Consequently, the injected DNA can be transmitted to offspring in a normal Mendelian manner. Mice carrying an exogenous gene in their genome are called "transgenic."

This technique was used to partially correct a mouse with a defect in its growth hormone production. By attaching a rat growth hormone gene to an active regulatory sequence (specifically, the promoter that normally directs the synthesis of metallothionein messenger RNA in mice), researchers obtained a recombinant DNA construct that actively produces growth hormone in the genetically defective mouse and in a number of its offspring. Although the level of growth hormone production was inappropriately controlled (that is, influenced by signals that normally regulate metallothionein synthesis), these experiments did show that microinjection can be used as a delivery system that can put a gene into every cell of an animal's body, that a genetic disorder can, as a result, be corrected, and that the correction can be passed on to the next generation of animals.

Why is the technique of microinjecting a fertilized egg not acceptable for use for human therapy at the present time? First, the procedure has a high failure rate; second, it can produce a deleterious result; and third, it would have limited usefulness. Microinjection has a high failure rate because the majority of eggs are so damaged by the microinjection and transfer procedures that they do not develop into live offspring. In one recent experiment involving microinjection of an immunoglobulin gene into mouse eggs, 300 eggs were injected; 192 (64%) were judged sufficiently healthy to be transferred to surrogate mothers, only 11 (3.7%) proceeded to live birth, and just 6 (2%) carried the gene. These results are from a highly experienced laboratory in which thousands of identical eggs from the same hybrid cross of inbred mice have been injected over several years. The mice were chosen because they gave the best results for gene transfer by microinjection. Attempts to microinject functional growth hormone genes into livestock eggs met with several major biologic and technical problems before being accomplished. Successful gene transfer by microinjection of human eggs, without a long period of trial and error experimentation, is extremely unlikely.

Second, microinjection of eggs can produce deleterious results because there is no control over where the injected DNA will integrate in the genome. For example, the integration of an exogenous rabbit beta-globin gene in transgenic mice can sometimes occur at a chromosomal location that results in expression of the beta-globin gene in an inappropriate tissue, namely, muscle or testis. There have also been several cases reported where integration of microinjected DNA has resulted in a pathologic condition. Although there is no control over where exogenous DNA will integrate in any gene transfer procedure, the damaging effect caused by a harmful insertion site could be great when it occurs in the egg but may be negligible when it occurs in one or a few of a large number of bone marrow cells.

The third objection to microinjection of eggs is limited usefulness. Not only is it ethically questionable to experiment on human eggs because of the expected losses,
but even if "success" were obtained, it would be applicable primarily when both patients are homozygous for the defect. When the parents are both carriers of a recessive trait, only one fertilized egg out of four would result in an affected child. Because a homozygous defect cannot yet be recognized in early embryos, and because the procedure itself carries such a high risk, it would be improper to attempt any manipulation in this situation. Furthermore, most of the very serious genetic disorders result in infertility (or death before reproductive age) in homozygous patients. Consequently, there would be little use for the procedure even if it were feasible.

Even when the technical capability becomes available to attempt germ line gene therapy in humans, there are major medical and ethical concerns to consider. The medical issues center primarily around the question, Will the transmitted gene itself, or any side effects caused by its presence, adversely affect the immediate offspring or their descendants? Because in this case one must study several generations of progeny to obtain answers, it will clearly take longer to gain knowledge from animal studies on the long-term safety of germ line gene therapy than on somatic cell gene therapy. Germ line therapy deserves careful ethical consideration well in advance of the time when the technical capability for carrying it out arrives. In a previous article, I have provided a discussion of the ethical issues involved in germ line gene therapy.

Enhancement Genetic Engineering

The third use of genetic engineering, enhancement genetic engineering, is considerably different in principle from the first two. This is no longer therapy of a genetic disorder; it is the insertion of an additional normal gene (or a gene modified in a specific way) to produce a change in some characteristic that the individual wants. Enhancement would involve the insertion of a single gene, or a small number of genes, that code for a product (or products) that would produce the desired effect—for example, greater size through the insertion of an additional growth hormone gene into the cells of an infant or fetus. Enhancement genetic engineering presents a major additional scientific hurdle, as well as serious new ethical issues.

The scientific hurdle to be overcome is formidable. Until now, we have considered the correction of a defect, of a "broken part," if you will. Fix the broken part and the human machine should operate correctly again. Replacing a faulty part is different from trying to add something new to a normally functioning system. To insert a gene in the hope of improving or selectively altering a characteristic might endanger the overall metabolic balance of the individual cells as well as of the entire body. Medicine is a very inexact science. Every year new hormones, new regulators, and new pathways are discovered. There are clearly many more to be discovered. Most impressive is the enormously intricate way that each cell coordinates within itself all of its thousands of pathways. Likewise, the body as a whole carefully monitors and balances a multitude of physiological systems. Much additional research will be required to elucidate the effects of altering one or more major pathways in a cell. To correct a faulty gene is probably not going to be dangerous, but intentionally to insert a gene to make more of one product might adversely affect numerous other biochemical pathways.

We possess insufficient information at present to understand the effects of attempts to alter the genetic machinery of a human. Is it wise, safe, or ethical for parents to give, for example, growth hormone (now that it is readily available) to their normal sons in order to produce very large football or basketball players? Unfortu-
nately, this practice is reported to take place in this country now. But even worse, why would anyone want to insert a growth hormone gene into a normal child? Once it is in, there is no way to get it back out. The child’s reflexes, coordination, and balance might all be grossly affected. In addition, even more serious questions can be asked: Might one alter the regulatory pathways of cells, inadvertently affecting cell division or other properties? In short, we know too little about the human body to chance inserting a gene designed for “improvement” into a normal healthy person.

Eugenic Genetic Engineering

The fourth level is “eugenic” genetic engineering. This area has received considerable attention in the popular press, with the result that at times unjustified fears have been produced because of claims that scientists might soon be able to remake human beings. However, traits such as personality, character, formation of body organs, fertility, intelligence, and physical, mental, and emotional characteristics are enormously complex. Dozens, perhaps hundreds, of unknown genes that interact in totally unknown ways probably contribute to each such trait. Environmental influences also interact with these genetic backgrounds in poorly understood ways. With time, as more is learned about each of these complex traits, individual genes that play specific roles will be discovered. Undoubtedly, disorders that are caused by defects in these genes will be recognized. Then, somatic cell gene therapy could be employed to correct the defect. But the concept of “remaking a human” (i.e., eugenic genetic engineering) is not realistic at present.

Conclusion

In summary, somatic cell gene therapy for human genetic disease should be possible in the very near future. The scientific basis on which this new therapeutic approach is founded has been thoroughly documented in a number of publications, as has the ethical justification for its use. Germ line gene therapy is still in the future, but the technical ability to carry it out will almost certainly be developed. Society must determine if this therapeutic option should be used. Enhancement genetic engineering should also be possible, and its medical and disturbing ethical implications need continuing discussion. Eugenic genetic engineering, in contrast, is purely theoretical and will, from a practical standpoint, be impossible for the foreseeable future. The topic is valuable for reflective thinking but not for scientific discussion.

Many of the fears generated by some articles in the popular press that discuss “gene therapy” or “genetic engineering” are unfounded. Insertion of single functional genes should soon become possible, but claims that new organs, designed personalities, master races, or Frankenstein monsters will be created can be given no credence in the light of what is currently known. Even so, we should be concerned about the possibility that genetic engineering might be misused in the future. The best insurance against possible abuse is a well-informed public. Gene therapy has the potential for producing tremendous good by reducing the suffering and death caused by genetic diseases. We can look forward to the day when, with proper safeguards imposed by society, this powerful new therapeutic procedure will be available.

Is there a place for initiating gene therapy in the fetus rather than waiting for the child to be born? There are a number of diseases, particularly involving the central nervous system, in which irreversible damage may already have occurred by the time of birth. If a vector could be developed that could safely be inserted into the central nervous system directly, or that could bypass the blood–brain barrier following its
introduction into the fetal circulation, then it might be possible to salvage those fetuses that are now doomed to irreversible damage before birth. The first step would be to demonstrate that in-utero bone marrow transplantation from a compatible donor would be beneficial to a fetus, for example, one that would be known to have Lesch-Nyhan disease. I encourage obstetricians to consider this therapeutic possibility.

References