Gene therapy

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Gene therapy is not yet possible, but may become feasible soon, particularly for well understood gene defects. Although treatment of a patient raises no ethical problems once it can be done well, changing the genes of an early embryo is more difficult, controversial and unlikely to be required clinically.

In itself, genetic manipulation is nothing new; in fact, some anthropologists mark the dawn of civilization from the point at which man began to use selective breeding for crops and domestic animals. Eugenics, as applied to humans, was a powerful ideology during the first thirty years of this century, and appealed to a surprising diversity of geneticists, of all political persuasions. Only the rise of totalitarianism during the 1930s ended this enchantment, making gene therapy the highly charged and emotive phrase that it is today.

Why has human genetics again become the focus of public and scientific attention? The reason is that, largely as a result of recombinant DNA technology, genes have ceased to be theoretical concepts and have become DNA sequences, to be isolated, studied and changed in the laboratory. It is now *relatively* routine to isolate a single gene coding for a particular protein from the several million 'gene-size bits' of DNA in the nucleus of every human cell, and to examine its functions. More important, it is possible to isolate and compare the equivalent genes from different individuals, making it feasible to identify abnormalities caused by mutations of the DNA. Once a serious gene defect can be identified in an individual, two possibilities arise. If the identification is made during the first few weeks after conception, the birth of affected individuals can be prevented, or, in theory at least, an attempt can be made to replace the defective gene by a functional one. While the former is an acceptable and effective practice in many societies, there remains much interest in the prospects and problems of the therapeutic approach.

Diseases for therapy?

The most obvious human genetic diseases which are candidates for potential gene therapy are those in which a single known gene does not function properly. In a few such diseases, altered DNA sequences have been cloned into bacterial plasmids. These can be used to identify whether an individual is homozygous for the disease or a carrier, and are disease-specific cloned probes. In this category are sickle cell anaemia and thalassaemia — in both of which the globin genes are defective — growth hormone deficiency, some of the

collagen disorders and Lesch-Nyhan syndrome in which there is an absence of the purine synthesis enzyme hypoxanthine phosphoribosyl transferase. In the near future, haemophilia (a defect of blood clotting factor VIII) and phenylketonuria will be added to this list, since attempts to isolate a gene probe are now being made in several laboratories. Further away, but equally interesting from the point of view of research (and the patients), is the possibility of determining the genetic defect of diseases such as Duchenne muscular dystrophy and Huntington's chorea; in these cases no defective gene product has yet been identified, but recombinant DNA technology allows one to bypass the product and hunt directly for an abnormal gene.

Single gene defects are important in clinical medicine. At present, in developed countries, they account for a high paediatric health-care burden — this is because they are chronic and often appear at birth, and may require replacement therapy with special diets or expensive natural products, such as blood clotting factor VIII or whole blood. In parallel with this clinical, economic and social perspective, there is also the personal problem faced by each family with a handicapped child. According to a recent World Health Organization report, there are over one hundred million carriers for potentially lethal inherited blood diseases such as sickle cell anaemia and thalassaemia, and approximately 200,000 affected children die each year from this single set of gene defects. In Britain, serious single gene defects (cystic fibrosis, muscular dystrophy and haemophilia are the most common) affect just under 1% of children.

Termination or gene therapy?

It is now possible to carry out antenatal diagnosis for some single gene defects (and also for chromosomal defects such as Down's syndrome, as well as for neural tube defects), and to offer the parents a termination of pregnancy if the fetus is affected. It may shortly be possible to do this during the first 2 months of pregnancy, rather than after 18 weeks as at present, by using samples of fetal chorion. As the number of disease-specific DNA probes increases, antenatal diagnosis by DNA analysis or linkage should become available for all single gene defects¹.

Why, then, even consider gene therapy for a life-threatening or handicapping inherited disease? Most communities at risk accept antenatal diagnosis and abortion of affected fetuses at the moment, as being better than having a severely handicapped child². This is true even in countries where one might expect a resistance to abortion, provided that the community knows the disease and its consequences. But there are a few families (rather less than one might expect) who refuse termination, are misdiagnosed or who do not come for diagnosis in time because of a failure on their part or on the part of the system. Also, there is a range of single-gene inherited diseases that require a lot of clinical attention but are perhaps not so handicapping that the families (and society) regard termination of pregnancy as acceptable (haemophilia, or phenylketonuria, might be in this group in some countries). Although the decision to elect abortion rests with the parents, it does so in a social and legal context determined by all of us as to the degree of handicap that is compatible with a normal life for the child or the family.

The route to gene therapy

If there is an identifiable need and demand for gene therapy (and the extent to which this is so will be discussed later), can it be done? At the moment, the answer is *unequivocally* no. This is an extremely important point; no gene replacement that is meaningful in the context of human genetic disease has yet been achieved even in model systems with animals.

Nevertheless, the way in which gene therapy might be carried out either on early embryos or on individual patients is clear in outline, from research now in progress, much of which was summarized at the meeting which inspired this article³.

Patient therapy

Gene therapy of a patient must involve the insertion of a functional human gene into the relevant tissue, where it can respond to the normal physiological stimuli of the body. First, the defective gene must be identified and a normal counterpart isolated. A method must be found to put the normal gene into a cell where it can function (the primitive red blood cell in the case of sickle cell anaemia, for instance, or the liver for phenylketonuria). To obtain correct gene action, it may be necessary to put it into the correct site on the host cell chromosome, or even to delete the defective gene. It is our inability to obtain correct gene function when DNA is put into a cell, and the fact that few inherited diseases affect only a single tissue such as bone marrow, that makes gene therapy impracticable at this time.

It is only 5 years since the first cloned human genes, those for globin, were isolated. Today, it is a relatively routine matter to isolate any gene when there is a specific antibody, a portion of the amino

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acid sequence of the protein encoded by the gene (for reverse synthesis of a bit of DNA using the coding rules, which can be used to pair with the natural gene), a tissue that selectively expresses the gene or a biological or drug selection assay for the gene. Each of these techniques has been used during the past year to identify genes that are expressed only at low levels in human tissues.

An isolated, cloned gene can either be directly injected into a cell or be carried in by a virus to which it has been linked by recombinant DNA techniques. Once in the cell, it may become integrated into the nuclear DNA, or remain free in the cytoplasm as a self-replicating, extrachromosomal element. In either case, it is usually transcribed to give RNA at the level of only a few copies per cell, as compared with the several thousand often achieved for endogenous genes. Translation may also be inefficient. Inserted genes are more stable if they are attached to another gene which can be selected, perhaps by drug resistance, and much effort is going into making virus-type gene vehicles with such selective markers which also cause the gene to be inserted into specific active sites on the chromosome.

Embryo therapy

Other experiments have attempted to put genes into early embryos to study their integration and inheritance. So far, the experimental aims have been academic rather than practical, but there is no reason in principle that this approach to gene therapy would not work, in conjunction with *in vitro* fertilization. Indeed, it might well have a greater chance of practical success than treating a particular tissue, but it also raises greater ethical problems because it would affect not only the individual but also his or her offspring.

Genes can be inserted into embryos with viruses, or directly by microinjection. In either case, a fertilized egg (from rabbit or mouse) takes up the new genes and integrates them in a random number of linked copies into its own DNA. The site of integration appears to be random, and in most cases so far, the integrated genes have been inactive. But in a few animals, genes inserted at the embryonic stage seem to have been switched on in a tissue-specific way. The best evidence so far concerns the gene for metallothionein, which binds to heavy metals. Even in this case, and certainly for globin, it is not clear that the tissue where the gene is expressed is always the right one.

Such new genes have been inherited in a normal way through at least three generations. (Note that this is so even though the inserted genes come from a species other than that of the embryo; the animal cannot tell the difference once integration into the chromosome has occurred, but the experimenter can — whence the mixing of species). These experiments have so far all been carried out

on mouse and rabbit embryos, and early human embryos available from work on *in vitro* fertilization are not known to have been used.

In summary, it is possible to isolate normal genes, to put them into mammalian cells at any stage, including the fertilized embryo, and to obtain gene function in a random way and at a low level. However, it is not yet possible to ensure that the 'therapeutic' newly inserted genes function under normal control in the animal, in time, space or quantity.

Is there a need?

To carry out gene therapy on an early embryo, one must be sure that it is affected by the disease — there is only a 25% chance that this will be true for most embryos at risk, and it might be thought that the parents, once the diagnosis has been made, would prefer to start conceiving again than consider gene manipulation of the embryo. Frankly, the prospect of gene therapy at the embryonic stage strikes me as ridiculous as a form of clinical therapy for a couple who have a 75% chance of a normal child (100% if they accept antenatal diagnosis) using the most popular, acceptable and enjoyable ways of having children that have been in vogue for many years without help or advice from molecular biologists. The correction of a disease by gene therapy will be worthwhile only if there is no other, simpler and more effective technique available.

On the other hand, treatment of tissue from a patient with a genetic disease raises few ethical problems, but is even more complex. The tissue must be accessible for isolation or transplantation, the gene must target to a region of the chromosome under appropriate control, and this is best done during early infancy, when the immune rejection system is still not fully developed. This must be balanced against the other options of antenatal diagnosis and conventional postnatal therapies, both areas where improvements are occurring rapidly.

The thalassaemia model

Consider thalassaemia, one of the most common single gene defects, about which we know a great deal. This can be diagnosed antenatally by fetal blood sampling and sometimes by gene analysis. Carriers are usually detected in school, before they start having children. In communities at high risk such as Sardinia, Cyprus and the relevant immigrant communities in Britain, the incidence has fallen to next to nothing by a combination of screening and antenatal diagnosis. The communities involved want and demand healthy children; at the moment this means accepting, reluctantly, antenatal diagnosis and termination of affected pregnancies.

Even in countries best equipped for diagnosis, a few cases are born each year. In the Cypriot community in Britain, there were 2 known thalassaemics born last year,

compared with about 30 who would have been born without antenatal diagnosis. There is a special problem in the United States, where the campaign against abortion has led to campaigns against antenatal diagnosis even for crippling disease. So even in the most advanced countries, there are still some cases of thalassaemia. Are they candidates for gene therapy?

At first sight the prospect looks attractive. The disease is well understood, the normal gene is available and widely studied and its position in the chromosome is known. Perhaps most important, the tissue affected by the disease, bone marrow and blood, is available and can be treated *in vitro* and transplanted back into a patient. This treatment could be carried out after birth, and without the additional problems of dealing with embryos and altering their genetics. However, even in this best case, problems will arise, for the proportion of early precursor red blood cells in the marrow that could be taken from the patient, treated and reintroduced, is only a tiny proportion of the total stem cell population, and marrow transplantation still involves major risk.

Other inherited diseases

In genetic diseases other than those affecting the blood, many tissues are affected. One obvious example is cystic fibrosis, the most common single gene defect in Britain, where lungs, pancreas, gut and sex organs can all be damaged to a variable extent. Even with dramatic advances in transplant therapy, it is unlikely that the removal of several tissues for individual gene insertion will become a realistic approach. In the absence of a 'target tissue', 'embryo therapy' has to be the chosen route, and for the foreseeable future this will remain unrealistic even if possible.

The entire question of gene therapy must also be seen in the context of rapidly changing biological and clinical knowledge. Personally, I think that there may be occasional cases where gene therapy is indicated (always in the future, when it can be done), and that in these cases we may well alter the inheritance ('genotype') of the individual as well as the way his body works ('phenotype'). This might be true for a period of a few years but I am certain that eventually far more sophisticated methods of treatment, probably involving therapy rather than gene alteration, will be developed.

Polygenetic disease

In developed countries, the major health hazards (apart from those due to growing old) — coronary heart disease, hypertension, early psychotic illness, diabetes, some forms of cancer — have both genetic and environmental components, perhaps in roughly equal amounts. Indeed, there is doubtless a genetic component to our general physical

and mental make-up, although no one knows yet how important it is compared with environment. There is popular concern that gene therapy will be applied in this area, to alter the vigour, personality or 'intelligence' — whatever that may be — of an individual. The Council of Europe recently reflected this in a recommendation which requested provision "for explicit recognition in the European Human Rights Convention of the right to a genetic inheritance which has not been artificially interfered with, except in accordance with certain principles which are recognised as being fully compatible with respect for human rights . . .".⁴

Such fears clearly strike a deep chord of response. Certainly, the hereditary factors in complex diseases such as diabetes and hypertension will be understood very much better as a result of research on isolated human genes. It is now possible to analyse, chromosome by chromosome, gene by gene, the components that make up any measurable inherited trait⁵. It has been postulated that a remarkably small number of cloned genes could 'cover' the entire human DNA with markers, and this seems to be true in practice⁶. The human gene map should be completely defined during the next 10 years.

Consider any serious illness, such as coronary heart disease, common in the community, a major killer of men aged between 30 and 50 in developed countries, with a large hereditary factor in this age group. There are very few cases where a single gene determines the risk; it is usually spread between several, especially those that determine the level of circulating cholesterol in the blood. Eventually, however, it should be possible to determine those at genetic risk of early heart attack soon after birth using DNA analysis.

Would gene therapy be possible in such cases? Since we know that many genes contribute to a healthy heart, it is unlikely that changing any one of them would lead to a dramatic physiological effect on the individual. Introducing genes would be difficult, and difficult to justify in the absence of a certainty of serious disease. Knowledge of the genetic factors is more likely to lead to new pharmacological approaches, and to prevention, than to gene therapy.

When we come to such vague concepts as 'aggression' or 'intelligence' or 'good looks', we are off in the land of the fairies. If there are any inherited determinants for these attributes, we are so far from understanding, isolating or measuring them that it is barely worthwhile even considering that an attempt at gene therapy could be made with any prospect of success.

Ethics

Given that it is unlikely that gene therapy will be attempted on any but the smallest scale, and even so not for some time, is it necessary to discuss the ethics of gene therapy at all? I think so. There must be

two clear starting points, both of which are fundamental to most humanistic and religious views. The first is that any person, whether well or ill, deserves respect as an individual. This concept underlies the prohibition on experimentation on patients for its own sake — experimental procedures can be carried out only when there is hope that the patient, or his immediate family, will benefit, unless the procedure involves minimum risk (when full consent is still required). Patients, however ill they may be, cannot be used as laboratory animals, because this is a denial of fundamental humanism. This is why there are special safeguards for those at high risk — the psychiatrically ill and mentally retarded, the terminally ill and long-term prisoners.

When, in a much-publicized incident in 1980, Dr Martin Cline of the University of California at Los Angeles attempted to treat marrow from two patients, one in Italy and one in Israel, with normal globin genes, and then carried out a limited marrow self-transplant, he violated this precept (see ref. 7). Therefore, Cline's experiments were *fundamentally* unethical. His own work on mice showed that there was no basis for hope that globin gene insertion into marrow cells could give clinical benefit at that time. The thalassaemic patients were irradiated at a high level to clear the marrow in the long bone of one leg, a procedure of some risk, and they and their families were given hope that the gene therapy might help them in their fight for survival. It is unacceptable that patients should be misled in this way, and certainly makes it more difficult for medical research workers to argue that our house is in order ethically.

Cline can also be criticized for *procedural* reasons — he carried out experiments on patients abroad which he could not perform in his own hospital in Los Angeles, in countries with inadequate ethical committee control, and he did not notify authorities that recombinant DNA was used. However, these breaches, although real and of concern to me — the clinicians involved in Italy and Israel must examine their own consciences and decide whether they behaved correctly and with full knowledge of the proposed treatment — are far less important than the fundamental breach of ethics discussed above. It should also be noted, lest we in Britain become too smug, that hospital ethical committees are not legally required in Britain, and in the many hospitals where they do not exist, there is little effective control over experiments on patients apart from a reliance on the good sense and judgement of the doctor involved.

The second underlying ethical 'rule' which most (but not all) people would accept is that the medical profession, as a caring profession, should do all it can to prevent serious handicap. Scientists who work with clinicians share this rather difficult obligation, and society has

decided that part of it is that a termination of pregnancy before approximately 5 months is allowable if the child would suffer a serious handicap. These are shifting sands, and the definition of 'serious handicap' is a social one, and rests with the community and the family at risk, and only secondarily with the doctor (and even less with the scientist).

It is often asked whether gene therapy poses long-term genetic problems to human inheritance. Naturally, this could arise only were the early embryo treated, so that the newly introduced genes are inherited; treatment of a patient's marrow or liver could not lead to genetic alteration. The human genome contains many tens of thousands of genes coding for polypeptides, and many hundreds of thousands of pieces of DNA of unknown function. Each of us 'normals' carries 20 or so potentially lethal recessive genes, from whose lethality we are protected by also carrying a healthy dominant version of the same gene. Our children will inherit some recessive lethal genes from each parent, but since our mates almost always carry a different set of deleterious genes, inherited disease is unlikely. Gene therapy, at worst, could inadvertently introduce an extra recessive lethal gene to an individual, increasing the load from about 20 to 21, in return for which a person who would otherwise be chronically ill, becomes healthy and a normal member of the community. From the point of view of the clinician there seems to be no fundamental ethical problem here.

Conclusions

The ultimate decisions as to whether and how we use gene therapy will rest with the community. Research with model systems and cell culture will continue in any case; these are well established and have other obvious benefits both to science and medicine. They will explain how normal and abnormal early embryonic development occurs, what causes miscarriage and chromosome defects, and the effects of environmental agents such as rubella or thalidomide.

Gene therapy is clearly not on the agenda for next month or next year. In my view, experiments with patients are clearly unethical at this time. When gene therapy is available, there must be a clear distinction between treatment of a serious disease in a way that affects only the patient himself, and treatment of an early embryo so that its inheritance is altered. However, gene therapy of both types will be possible in the future, and it should be considered now, before the headlines break on us all.

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