

Gene Therapy

Treatment of disease by introducing healthy genes into the body is becoming feasible. But the therapy will not reach its full potential until the genes can be coaxed to work throughout life

by Inder M. Verma

One infant in every hundred is born with a serious genetic defect. Usually the damage becomes evident in childhood. All too often, it gives rise to physical or mental abnormalities, pain and early death. Of the more than 4,000 known inherited disorders, most lack fully effective therapies.

It is no wonder, then, that scientists have long imagined curing heritable ills by introducing healthy genes into patients. Advances in recombinant DNA technology, which have made possible the isolation of many genes, and new insights into gene regulation are beginning to make this once impossible notion seem feasible.

Indeed, the first federally approved clinical trial of a gene therapy for a genetic disease began this past September. R. Michael Blaese, W. French Anderson and their colleagues at the National Institutes of Health (NIH) are introducing the gene for the enzyme adenosine deaminase (ADA) into children suffering from a rare condition known as severe combined immunodeficiency (SCID). Derangement of this gene debilitates the immune system and is responsible for about 25 percent of all cases of SCID.

The approach of the NIH group requires repeated treatments throughout life, and so it is not a cure. Still, the trial could represent the start of a new era in medicine. The current pace of research suggests that by the turn of the next century clinical trials of gene therapies may be under way for any

of a number of diseases—inherited and otherwise.

Genes can be transferred either into germ cells (sperm, eggs or early embryos) or somatic cells (those not destined to become sperm or eggs). Yet germ-line therapy is not an option for the foreseeable future, in part because the new genes would be passed from generation to generation, a prospect that raises profound ethical concerns.

For instance, should therapy be applied simply to improve one's offspring, not only to prevent an inherited disease? Who would be empowered to decide? Is society willing to risk introducing changes into the gene pool that may ultimately prove detrimental to the species? Do we have the right to tamper with human evolution? The prospect of somatic cell therapy is less troubling, mainly because it would affect only the treated patient.

The most promising candidates for somatic cell therapy are disorders caused by impairment of a single gene that has been isolated and cloned and so is available for transplant. These diseases should be simpler to correct than those caused by multiple genes or by such global disturbances as the loss or addition of whole chromosomes. (Normally, human cells carry one set of 23 chromosomes inherited from the mother and a corresponding set from the father. Every chromosome consists of a long stretch of DNA and includes thousands of genes.)

In the ideal world, the diseases would be cured for life by one treatment, with no side effects. And gene insertion into a chromosome in a target somatic cell would be site specific: in what is called homologous recombination, the healthy, or "therapeutic," gene would exactly replace the damaged copy. Targeted insertion increases the probability that a therapeutic gene will function correctly. It also reduces the likelihood that random insertion will activate a quiescent oncogene (a

cancer inducer) or inactivate a cancer suppressor.

In reality, investigators have found it extremely difficult to control the fate of DNA introduced into cells. For every gene spliced into the correct place, more than 1,000 fit randomly into the genome (the total DNA in a cell). Work by Mario R. Capecchi of the University of Utah suggests that the obstacles to site-specific gene delivery are great but surmountable. Meanwhile many laboratories, including my own at the Salk Institute in La Jolla, Calif., are concentrating on developing gene augmentation therapy, in which a healthy gene replaces the product of a missing or defective gene but does not physically replace the flawed DNA itself.

Augmentation can be helpful when a genetic derangement results in little or no production of a protein. (Each gene encodes, or carries instructions for, a single protein.) Low production occurs when mutations hamper the activity of both the maternal and paternal copies of a gene or when a hobbled gene is inherited on a male's only X chromosome. (The cells of males carry one X and one Y chromosome; those of females carry two X chromosomes.)

On the other hand, augmentation therapy might not be of much help when a mutation yields overproduction of a protein or the synthesis of a destructive substance, as is the case in sickle cell anemia. To correct those kinds of disturbances, therapy would often have to include delivery of both a healthy gene and one capable of inactivating the mutated version.

For now, most scientists interested in gene augmentation are planning to remove cells from patients, introduce a therapeutic gene and return the altered cells to the subject. Some day, however, physicians may directly inject patients with genes linked to substances that will deliver those genes to specific target cells.

Fortunately, genetic flaws do not necessarily have to be corrected in all of

INDER M. VERMA is professor of molecular biology and virology at the Salk Institute in La Jolla, Calif., and adjunct professor of biology at the University of California, San Diego. He joined the institute in 1974 after earning a doctorate in biochemistry at the Weizmann Institute of Science in Israel and completing postdoctoral studies at the Massachusetts Institute of Technology.

the body's trillions of cells in order for therapy to work. First, even though every somatic cell in an individual carries identical chromosomes, certain genes function only in a single cell type. Treatment, then, could focus only on that type. Second, even when a genetic defect results in insufficient synthesis of a protein made in virtually every cell, many cells compensate for the loss. For instance, a flaw in the ADA gene affects most somatic cells to a degree but is devastating only to some constituents of the immune system.

Nontargeted delivery of genes into cells can be accomplished by chemical or physical means (transfection) or by viruses (transduction). In chemical approaches, one mixes many copies of DNA carrying the healthy gene with a charged substance—typically calcium phosphate, DEAE-dextran or certain lipids. Then the mixture is essentially dumped onto recipient cells. The chemicals disturb the cell membrane and transport the DNA into the interior.

The procedure is simple, but the efficiency of gene delivery is dismal. Usually only one cell in 1,000 to 100,000 integrates the gene of interest into its genome. A physician would have to obtain an impossible number of cells from patients to guarantee the appropriate alteration of the millions required for therapy.

I should point out that integration is not always crucial to gene expression (production of the encoded protein). Still, a gene that is integrated is likely to last longer in the cell. Further, it should replicate whenever the rest of the DNA does, as when a cell prepares to divide. The therapeutic gene would thus be inherited by the daughter cells and by their daughters and so on, thereby ensuring a supply of the product throughout a patient's life.

Physical methods include microinjection with a fine glass pipette and electroporation (the exposure of cells to an electric shock). The shock renders cells permeable to DNA in the surrounding medium, but it can also severely damage them. Microinjection can be ex-

remely efficient; perhaps one cell in five takes up the foreign gene permanently. Yet because only a single cell can be injected at a time, this tedious, labor-intensive approach is not suitable for therapeutic purposes.

The final strategy capitalizes on the native ability of viruses to enter cells, bringing their own genetic material with them. Many of these organisms have now been engineered to serve as vectors, or delivery vehicles, for gene transfer. Viruses can be grouped according to whether their genetic material is DNA or RNA. The two substances have important chemical differences, although both are built from units known as nucleotides and both include regulatory codes in addition to those specifying the sequences of amino acids in proteins.

Many DNA viruses that can accept foreign genetic material turn out to be severely limited in the number of nucleotides they can accommodate and in the range of cells they infect. Certain other DNA viruses are roomier but have so far proved unusable for var-



STERILE BUBBLE protected a boy named David, who suffered in the 1970s from severe combined immunodeficiency, or SCID, an inherited disorder in which the immune system is

profoundly impaired. SCID patients have better options today and may have more in the future: the first gene therapy approved for clinical trial aims to ease a form of the disorder.

ious reasons. Moreover, DNA viruses often do not splice their genetic material into the chromosomes of the cells they infect.

As is true of the DNA viruses, most RNA viruses are unsuitable for gene therapy, mainly because RNA, which cannot integrate into the DNA of human cells, is degraded rapidly. Varieties known as retroviruses are an exception. They actually convert their RNA to DNA in infected cells and insinuate the DNA into a chromosome. The integrated DNA then directs the synthesis of viral proteins. Retroviruses can entertain more foreign genetic material than some DNA viruses. They can also infect a broad spectrum of species and cell types.

For these reasons, retroviruses are the most promising gene-delivery systems studied thus far. Indeed, unless specified, all approaches to gene transfer discussed in the balance of this article are based on these vectors.

Retroviruses are, of course, not without obvious drawbacks. For instance, they can merge their DNA into a chromosome only in cells capable of actively dividing. Yet many cells do not normally divide—among them, mature neurons—and so they are not readily amenable to being genetically altered by retroviral vectors.

More disturbing is the possibility that retroviruses can cause cancer. The risk is extremely low for the species that have been considered as vectors, but it increases if the viruses are allowed to multiply in the body and spread from cell to cell. Consequently, a major challenge has been devising ways to stop the vectors from reproducing.

The efforts of several laboratories have together yielded at least one technique that seems to work well [see illustration on page 72]. The organisms produced by that method have a normal outer coat and contain all of the virus's proteins. The retroviral RNA, however, includes no instructions for synthesizing viral proteins. The therapeutic gene takes the place of those missing instructions.

The coat enables the viruses to enter cells and deliver the viral contents to the cell's cytoplasm. Then viral enzymes convert the RNA to DNA and help to fit that DNA into the genome of the host cell. But that is the end of the line for the virus.

Under normal circumstances, integrated retroviral DNA—called the provirus—would direct the synthesis of viral proteins and RNA, which would then assemble into clones of the original virus. In contrast, the altered retro-

virus, bereft of instructions for making viral proteins, produces no progeny. The virus essentially disappears from the cell, leaving behind only the foreign gene and nucleotide sequences that now serve merely to facilitate the expression of the gene.

Although retroviruses can infect many cell types, only certain target cells can be considered for genetic manipulation. The cells must be strong enough to withstand handling and capable of being removed from the body and returned with reasonable ease. In addition, they should be long-lived, surviving for months or years or preferably for the patient's entire life. Because bone marrow, skin and liver cells best meet these criteria, diseases that can be treated by manipulating these cells are among the most promising candidates for gene therapy.

The cells of the bone marrow, where blood is produced, can in theory be exploited to correct disorders caused by genetic flaws in red blood cells or in white blood cells (which are important in immunity). SCID caused by an ADA deficiency is but one of several inherited conditions affecting the immune cells; another is leukocyte adhesion deficiency, which involves the poor mobilization of white blood cells and leads to recurrent infections. Among the diseases associated with impaired red blood cells are the thalassemias, which reflect impairments in the genes encoding subunits of the hemoglobin molecule—the oxygen carrier in red blood cells.

Beta thalassemia was once expected to be the first disorder treated with gene therapy. Its history illustrates some of the problems that have beset the effort to develop gene therapy in general and therapy based on bone marrow cells in particular.

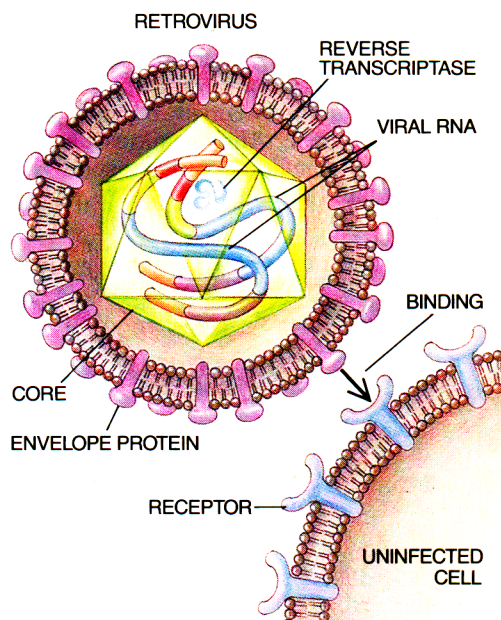
Red blood cells of patients stricken with beta thalassemia are deficient in beta globin, which in healthy individuals combines with alpha globin and iron (heme) to yield hemoglobin. Healthy cells regulate the activity of both genes precisely, ensuring that equal amounts of alpha and beta globin are made. The lack of beta globin gives rise not only to a deficit in hemoglobin production but also to a relative excess of alpha globin. This excess, in turn, hastens cell death and can cause severe anemia. Usually patients succumb to the disease by age 20, after years of pain and suffering.

This disease and other inherited blood disorders could probably be treated efficiently by delivering healthy genes to stem cells, the subset of cells

in the marrow that gives rise to the full spectrum of blood cells and replaces dead cells throughout a person's life. Stable introduction of a desired gene into a stem cell could guarantee the production of normal blood cells for as long as a patient lives.

Sadly, human stem cells are far from abundant and are virtually impossible to isolate. Researchers have therefore been forced to resort to a less efficient strategy: infecting enormous numbers of bone marrow cells with a therapeutic retrovirus in the hope that enough stem cells will be infected.

Studies of beta globin have supplied much of the evidence showing that the approach has at least some merit.



LIFE CYCLE of a retrovirus begins when the virus binds to (above) and enters (right) a cell and injects its genetic material (RNA) and proteins into the cytoplasm. Typical retroviral RNA includes three coding regions: *gag* (green), *pol* (blue) and *env* (purple), specifying, respectively, proteins of the viral core, the enzyme reverse transcriptase and constituents of the coat. It also has three noncoding domains—two at the tips (light orange) and another called *psi*, ψ (red). In the cytoplasm, reverse transcriptase converts the RNA into DNA, whose lengthened terminal domains, called long-terminal repeats (dark orange), influence the activity of viral genes and facilitate insertion of viral DNA into cellular DNA. The ensconced DNA (the provirus) directs the synthesis of viral proteins and RNA. The proteins then enclose the RNA, forming viral particles that bud from the cell.

For instance, several laboratories have shown that a human beta globin gene inserted into mouse bone marrow cells by retroviral vectors stays in the cells. And Richard C. Mulligan and his co-workers at the Whitehead Institute for Biomedical Research in Cambridge, Mass., have further shown that the human gene is expressed when such cells are implanted in mice.

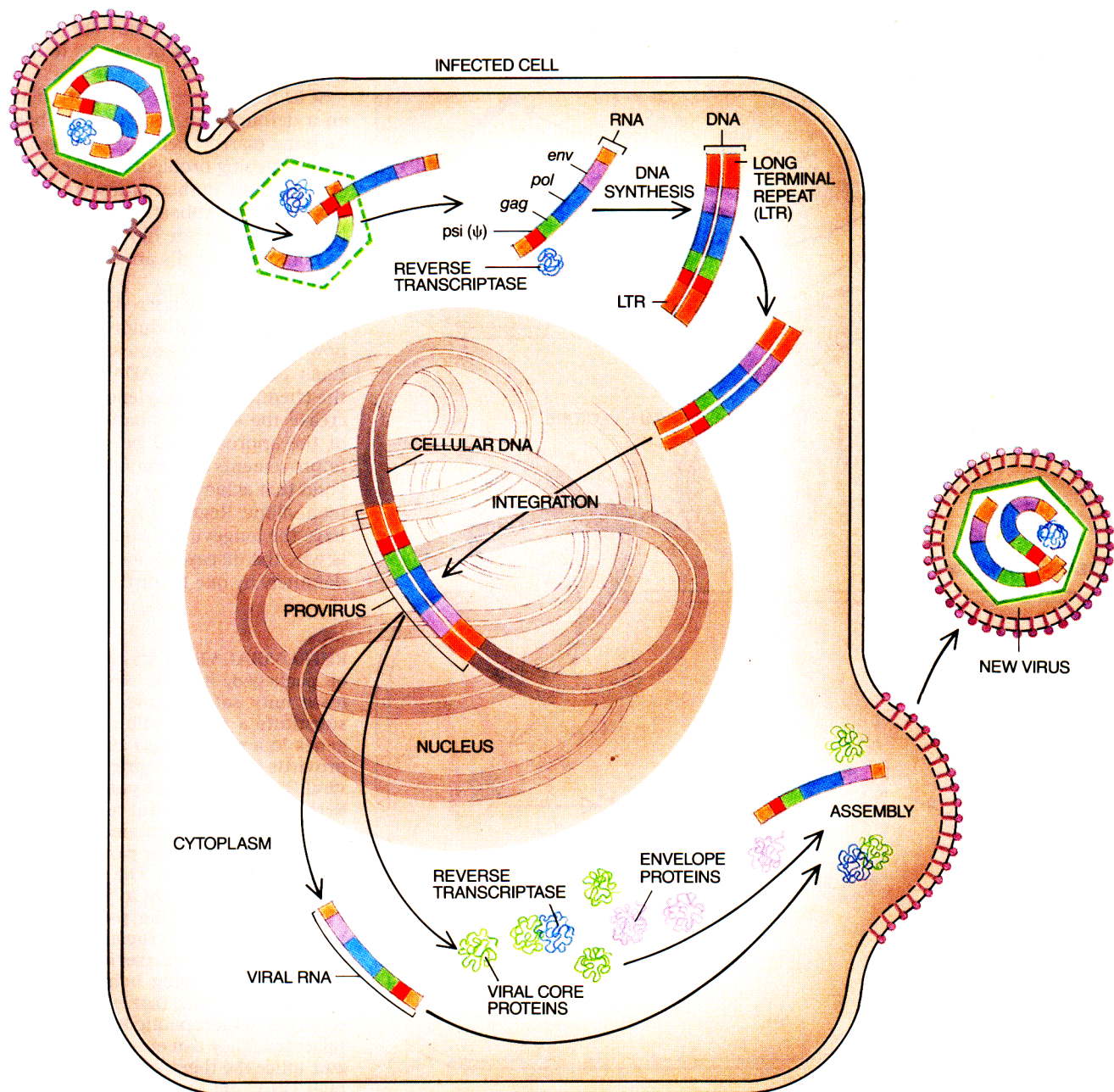
On the other hand, no one has been able to achieve significant levels of globin synthesis in recipient animals. This problem has been a major disappointment, but a discovery by F. G. Grosveld and his colleagues at the National Institute for Medical Research in London offers hope for a solution.

They identified distinct stretches of DNA, thousands of nucleotides apart from the gene itself, that in normal red blood cells dramatically boost the production of globin messenger RNA. Messenger RNA is transcribed, or copied, from DNA and is the template from which protein is made; hence, high levels of a messenger RNA indicate that the encoded protein is being produced in abundance. It seems reasonable to think that linking globin-specific enhancers to a globin gene in a retroviral vector might enhance globin synthesis in the body. Studies of this hypothesis are in progress.

In general, genetically altered bone marrow cells have yielded poor in vivo

expression of other genes as well. The problem must be resolved before gene therapy based on bone marrow cells can become a reality.

Along with an acceptable level of gene expression, one would hope for long-term activity. Recent findings relating to globin indicate that achieving prolonged expression of genes inserted in bone marrow may be less problematic than attaining high levels of protein synthesis. For instance, Chung L. Li and V. J. Dwarki in my laboratory have produced sustained, albeit weak, expression of the human beta globin gene in mice for at least a five-month study period—the equivalent of 15 to 20 years in a human being. The alpha



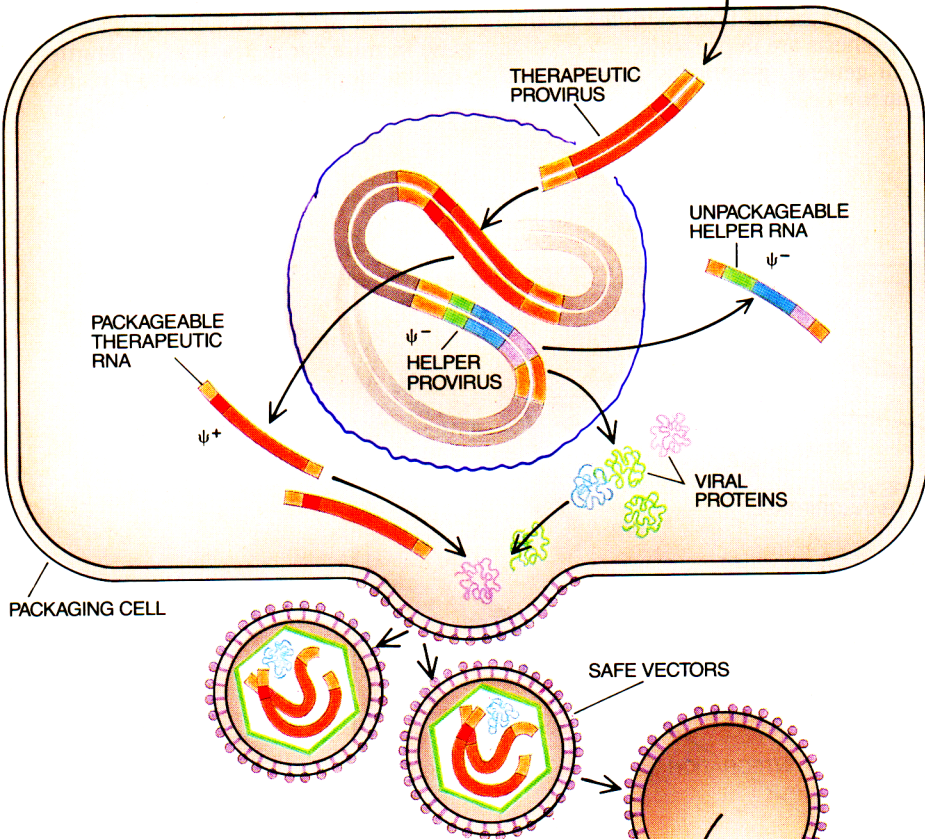
Other findings emerging from the work on beta thalassemia highlight the complexity introduced when correction of a disease requires precisely regulated expression of a therapeutic gene. For many disorders, including SCID, simply producing some amount of a missing protein is better than none. The same is not true for thalassemia. Because a relative excess of either alpha or beta globin can damage cells, the activity of a therapeutic globin gene must exactly mimic that of a normal version. Unfortunately, the mechanisms that control the activity of genes are understood only imperfectly—both for the beta globin gene and for most others. Discoveries are made constantly, however, and are helping improve the design of vectors for gene therapy.

SCID researchers at the NIH have taken a detour from gene therapy based on bone marrow cells, in part because of the ongoing problem of poor expression. Patients in their study are treated with a select subset of circulating *T* lymphocytes, white blood cells crucial to immunity. *T* cells are devastated by a lack of ADA.

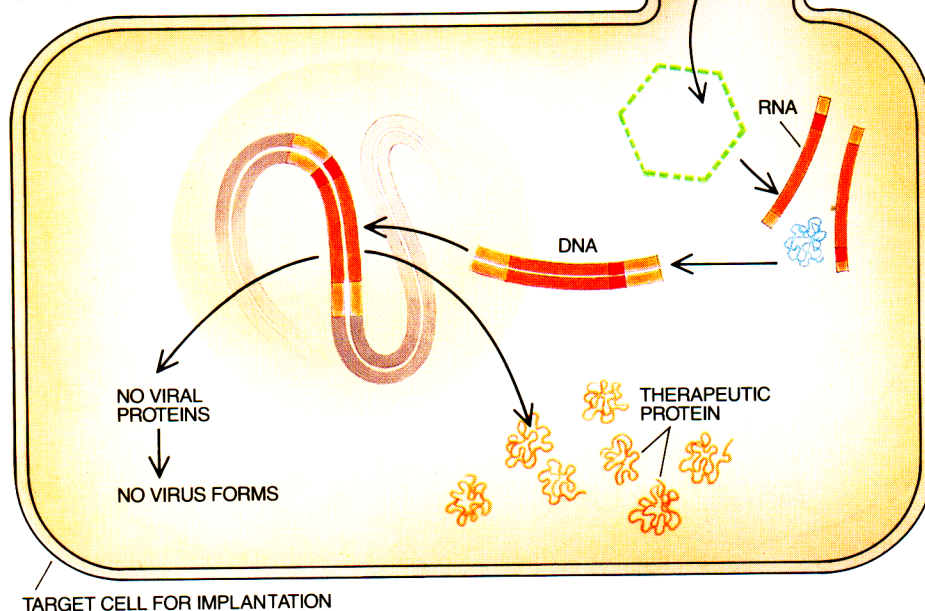
The retrovirally altered lymphocytes are infused into children who are now being helped somewhat by injections of PEG-ADA—ADA mixed with the chemical polyethylene glycol to increase the enzyme's half-life. Success of the approach will be measured by improvements in immune function beyond that achieved by enzyme replacement alone. Regrettably, *T* cells do not have the longevity of stem cells, which is why the disease cannot be cured indefinitely by one treatment.

RETROVIRAL VECTORS are assembled, or packaged, in cells designed to release only safe vectors. Investigators substitute a therapeutic gene for viral genes in a provirus (a) and insert that provirus into a packaging cell (b). The viral DNA directs the synthesis of viral RNA but, lacking viral genes, cannot give rise to the proteins needed to package the RNA into particles for delivery to other cells. The missing proteins are supplied by a "helper" provirus from which the psi region has been deleted. Psi is crucial to the inclusion of RNA in viral particles; without it, no virus carrying helper RNA can form. The particles that escape the cell, then, carry therapeutic RNA and no viral genes. They can enter other cells (c) and splice the therapeutic gene into cellular DNA, but they cannot reproduce.

b INSERT INTO PACKAGING CELL



c INCUBATE WITH TARGET CELLS



The availability of nongenetic treatments for SCID (including bone marrow transplantation) raises the general question of whether subjecting patients to highly experimental gene therapies is justified when alternatives exist. The prevailing opinion holds that such experimentation is acceptable if the risks are demonstrably low and if, on the one hand, a gene therapy promises to be significantly more helpful than existing approaches or, on the other, patients are ineligible for the established treatments. In the case of SCID, for example, not all patients have access to bone marrow from a tissue-compatible donor.

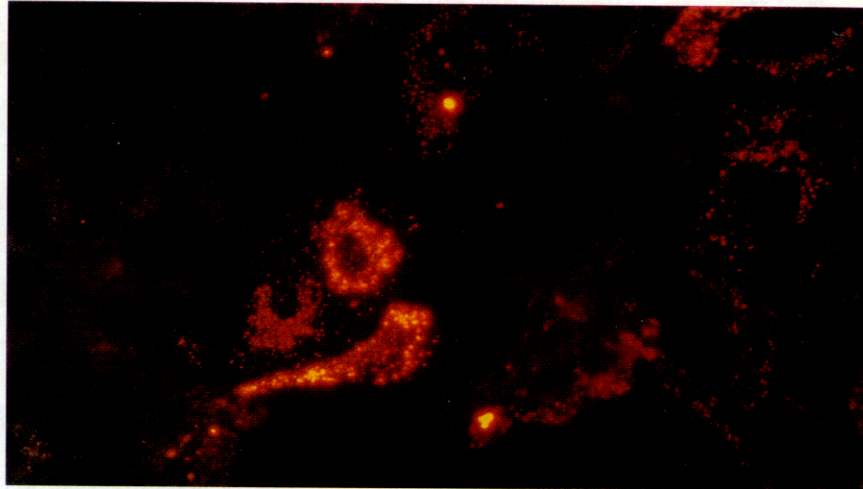
Genetic alteration of lymphocytes or bone marrow cells aims to correct defects in those same cells or their progeny. Skin cells, in contrast, are being studied for quite a different purpose: the synthesis and secretion of proteins that are normally made in one cell type but are ferried in blood plasma for use by other cells.

In principle, implants of skin cells could correct many disorders. These conditions might include hemophilia (caused by a lack of blood-clotting factors made in the liver) and diseases caused by insufficient production of particular hormones (for example, growth hormone). Certain disorders caused by deficient production of widely made proteins would also be candidates, if the tissues most affected by the deficiency could take up replacement proteins from the blood.

Fibroblasts, a constituent of the dermis (the lower layer of the skin), are best suited for therapy, which would involve implanting the altered cells back into the dermis. They are accessible and strong and able to multiply in the laboratory. Furthermore, they can secrete substances into the blood and would be easy to remove if necessary.

My laboratory has extensively studied the value of skin fibroblasts for treating the form of hemophilia caused by a lack of the liver product known as clotting factor IX. Our results underscore the great therapeutic potential of such cells.

In one of our studies, for instance, A. Dusty Miller, now at the Fred Hutchinson Cancer Research Center in Seattle, collaborating with George G. Brownlee and Don S. Anson of the University of Oxford, showed that fibroblasts could be induced to synthesize and secrete factor IX, even though they do not typically make that protein. (Whether the same will be true for all foreign proteins remains to be seen.) Furthermore, when Daniel C. St. Louis, Jonathan H.



LIVER CELLS from rabbits genetically deficient in the receptor for low-density lipoprotein (LDL) began to make the missing receptor (*bright regions*) after being altered to carry the receptor gene. The finding raises the possibility that a similar genetic disorder leading to excess serum cholesterol in humans might one day be treatable by gene therapy. James M. Wilson of the Howard Hughes Medical Institute Research Laboratories at the University of Michigan at Ann Arbor and J. Roy-Chowdhury of the Albert Einstein College of Medicine made the photomicrograph.

Axelrod and Raphael Scharfmann in my group used retroviruses to insert the human factor IX gene into fibroblasts and implanted the cells in the dermis of mice, the implants became highly vascularized and released the factor into the blood.

This study not only demonstrated that expression of factor IX in animals was possible, it also taught us an important lesson. About 15 days after the cells were implanted, the human factor disappeared from the blood of the mice. The recipients, it turned out, had mounted an immune response against the foreign human protein. The moral: gene therapy will probably be most successful in patients who make at least a small amount of a deficient protein; otherwise the immune system may become aroused against the product of an inserted gene.

We have also found some evidence to suggest that, unlike the bone marrow cells studied to date, fibroblasts may be able to produce enough of a selected product to correct disease. Extrapolation from data in mice indicates that an implant the size of a quarter should make enough protein to alleviate a factor IX deficiency in a human. In collaboration with Kenneth M. Brinkhous of the University of North Carolina at Chapel Hill, we expect to study the ability of fibroblast implants to correct hemophilia in dogs. If those experiments are successful, trials in humans would be justified.

Genetically altered fibroblasts might also be implanted in the brain to correct disorders in neurons. The brain is

notoriously hard to treat because many drugs that circulate in the blood are barred from the brain. Moreover, neurons cannot be removed for direct genetic alteration without consequence to the brain. Fibroblasts could in theory be engineered to secrete proteins for diffusion into nerve cells.

Preliminary results are encouraging. Fred Gage of the University of California at San Diego has shown that implants engineered to secrete nerve growth factor could stimulate neuronal growth in the rat brain. The regeneration occurred in the kinds of neurons whose decay is associated with memory loss in Alzheimer's disease, although the role of the factor in that disease has not been established. Similarly, implants that make levodopa (L-dopa), a precursor of the neurotransmitter dopamine, are under study in animal models of Parkinson's disease. No one knows exactly what causes Parkinson's, but a deficiency of dopamine seems to play a part. Exactly how long fibroblast implants can survive in the skin or brain is still being investigated.

Compared with bone marrow and skin cells, liver cells are a newcomer to the field of gene therapy. They could become important for the treatment of any number of genetic diseases caused by malfunctioning liver cells. Recently, for instance, Mulligan of the Whitehead Institute and James M. Wilson, then also at the institute, and, separately, Theodore Friedmann and his colleagues at San Diego succeeded in delivering the gene for

the low-density lipoprotein (LDL) receptor to liver cells and inducing them to make biologically active receptors in the laboratory. The cells came from Watanabe rabbits, which are genetically deficient in the LDL receptor—as are humans afflicted with familial hyper-

cholesterolemia, a condition that can lead to heart attacks.

The feasibility of directly injecting live Watanabe rabbits with complexes of the receptor gene and a protein that homes to the liver has also been studied. (Direct injection in humans would,

of course, avoid surgery to remove liver cells.) The encoded protein was detected in the body but, as was also true in the cell-culture study, was made only transiently. Longevity may yet be improved; investigation of liver cells is still in its infancy.

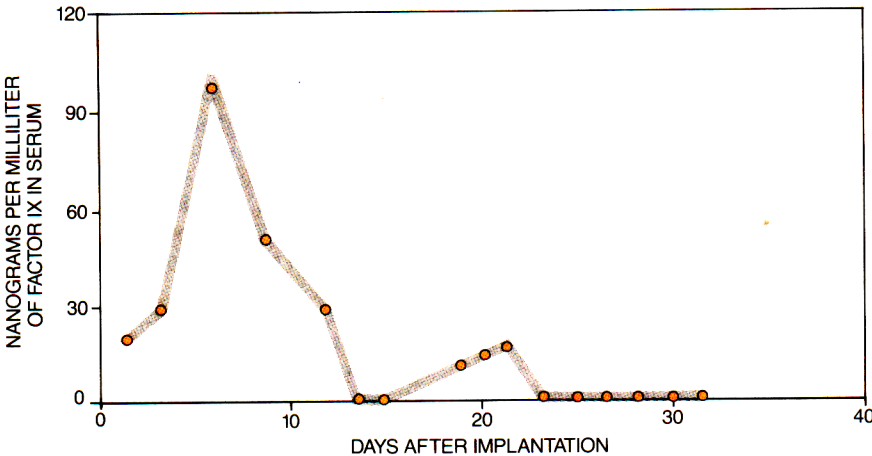
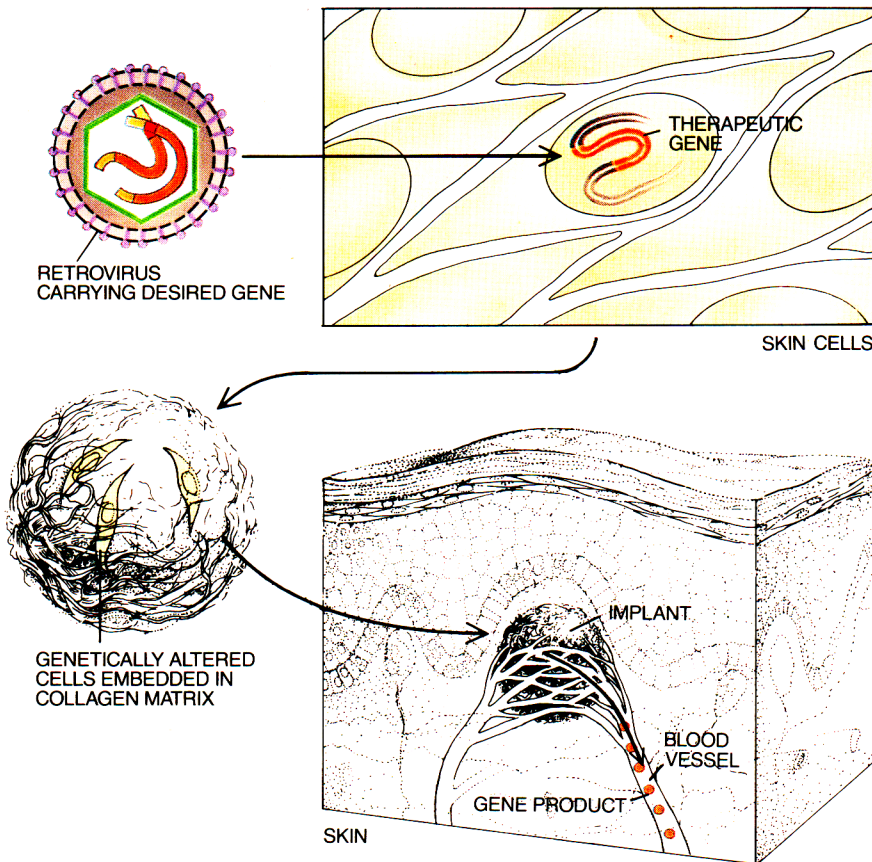
Although bone marrow, skin and liver cells are receiving the most attention, other types are also being considered. For instance, retroviruses can carry genes for secretory products into endothelial cells, which line the arteries. These cells have more intimate contact with the blood than do fibroblasts, and so they might deliver the products more quickly.

Researchers are also considering injecting a healthy gene encoding dystrophin (a structural component of muscle) directly into muscles of mice that have acquired a disorder akin to Duchenne's muscular dystrophy. There is reason to hope the genes will be expressed; other genes injected into muscles in live animals gave rise to proteins for several months, even though the DNA was not integrated into chromosomes. It may also be possible to treat cystic fibrosis, an inherited lung disorder, by packaging healthy genes in retroviruses that would be inhaled in an aerosol spray.

Gene therapy does not have to be limited to repairing the effects of malfunctioning genes. It can also add novel properties to cells to enhance their ability to combat disease.

For instance, Steven A. Rosenberg and his colleagues at the National Cancer Institute have demonstrated that lymphocytes taken from a patient's tumor and cultured with interleukin-2 (a T cell activator) can shrink some cancers. They now hope to increase the cancer-fighting powers of those tumor-infiltrating lymphocytes, or TILs, by inserting a gene encoding tumor necrosis factor, a potent immune-system molecule. The factor, which has anticancer activity, is not ordinarily made in T cells. Clinical trials are expected to begin soon [see "Adoptive Immunotherapy for Cancer," by Steven A. Rosenberg; SCIENTIFIC AMERICAN, May].

In more preliminary work, another group is trying to induce various cell types to produce CD4, a molecule found on T cells depleted by the AIDS virus. The virus enters the cells after a protein in its coat binds with CD4. A flood of CD4 molecules in the blood might serve as a decoy to keep the virus from interacting with the cells. Many other creative ideas for applying gene therapy are also being discussed, including coaxing endothelial cells to secrete factors that would pre-



SKIN CELLS carrying an inserted gene can be embedded in a collagen matrix and implanted in the dermis to deliver the gene's product to the blood (top). In one early experiment, skin fibroblasts containing the human gene for factor IX, a protein normally secreted by the liver to aid in blood clotting, became well vascularized in mice and secreted the human factor for approximately two weeks (graph). Much longer release of foreign proteins has now been achieved by fibroblast implants.

DISORDER	INCIDENCE	NORMAL PRODUCT OF DEFECTIVE GENE	TARGET CELLS	STATUS
Hemoglobinopathies (thalassemias)	1 in 600 in certain ethnic groups	Constituents of hemoglobin	Bone marrow cells (which give rise to circulating blood)	Globin production in animals receiving gene needs to be improved
Severe combined immunodeficiency (SCID)	Rare	Adenosine deaminase (ADA) in about a quarter of SCID patients	Bone marrow cells or T lymphocytes	Clinical trial of lymphocyte therapy for ADA deficiency is under way
Hemophilia A Hemophilia B	1 in 10,000 males 1 in 30,000 males	Blood-clotting factor VIII Blood-clotting factor IX	Liver cells or fibroblasts	Good chance for clinical trials (with fibroblasts) in next five years
Familial hypercholesterolemia	1 in 500	Liver receptor for low-density lipoprotein (LDL)	Liver cells	Animal studies are in early stages
Inherited emphysema	1 in 3,500	Alpha ₁ -antitrypsin (liver product that protects lungs from enzymatic degradation)	Lung or liver cells	Work is very preliminary
Cystic fibrosis	1 in 2,500 Caucasians	Substance important for keeping air tubes in lungs free of mucus	Lung cells	Aerosol delivery of gene directly to lungs is a theoretical possibility
Duchenne's muscular dystrophy	1 in 10,000 males	Dystrophin (structural component of muscle)	Muscle cells (particularly embryonic ones that develop into muscle fibers)	Work is preliminary. Nondystrophin genes injected into muscle have directed synthesis of the encoded proteins
Lysosomal storage diseases	1 in 1,500 acquires some form	Enzymes that degrade complex molecules in intracellular compartments known as lysosomes	Vary, depending on disorder	Most diseases would require delivery of gene into brain cells (a difficult task) as well as into other cell types

POTENTIAL CANDIDATES for the earliest gene therapies will be disorders caused by defects in a single gene that has been cloned. In general, physicians will remove cells from a patient, insert a healthy gene and return the cells to the body.

vent blood clots from forming in a patient's arteries after heart surgery.

The idea of introducing genes to correct heritable and other disorders is nothing less than revolutionary. Perhaps that is one reason why the field has progressed somewhat more slowly than was once expected. Modern creatures are the products of millions of years of evolution. One cannot expect that the initial stabs at inserting genes into cells will yield normal, stable expression easily.

Yet to cure diseases, investigators must find ways to ensure that therapeutic genes are expressed well and persistently in the body. Continually emerging clues, such as the importance of including particular enhancers with some genes in retroviral vectors, are beginning to point the way. Also needed are better methods for returning genetically altered cells (such as liver cells) to the body, ways of extending the survival of implanted cells, and techniques for isolating human stem

cells (to replace the bone marrow cells now being studied).

At the same time, the safety of retroviral vectors must be confirmed in extensive studies of both small and large animals, and efforts to incorporate added safeguards should continue. In spite of the advent of retroviral vectors that cannot replicate, there is still a chance they could cause cancer. Efforts to develop alternatives to retroviral vectors should be pursued further as well, as should research into site-specific gene delivery.

The goal of curing genetic diseases for life with a single, safe treatment is unquestionably worth the effort being put into it, but I must end with the reminder that gene therapy cannot correct all human disease. Most human afflictions are not genetic. They are environmental, caused by microbial infections that spread because of poor sanitation, polluted drinking water, malnutrition and other factors that are outside the scope of genetic engineering. Those diseases, too, deserve increased study.

FURTHER READING

- LINEAGE-SPECIFIC EXPRESSION OF A HUMAN β -GLOBIN GENE IN MURINE BONE MARROW TRANSPLANT RECIPIENTS RECONSTITUTED WITH RETROVIRUS-TRANS-DUCED STEM CELLS. Elaine A. Dzierzak, Thalia Papayannopoulou and Richard C. Mulligan in *Nature*, Vol. 331, No. 6151, pages 35-41; January 7, 1988.
- DISRUPTION OF THE PROTO-ONCOGENE *INT-2* IN MOUSE EMBRYO-DERIVED STEM CELLS: A GENERAL STRATEGY FOR TARGETING MUTATIONS TO NON-SELECTABLE GENES. Suzanne L. Mansour, Kirk R. Thomas and Mario R. Capecchi in *Nature*, Vol. 336, No. 6197, pages 348-352; November 24, 1988.
- AN ALTERNATIVE APPROACH TO SOMATIC CELL GENE THERAPY. D. St. Louis and I. M. Verma in *Proceedings of the National Academy of Sciences*, Vol. 85, pages 3150-3153; 1988.
- HUMAN GENE THERAPY. Eve K. Nichols and the Institute of Medicine, National Academy of Sciences, Staff. Harvard University Press, 1988.
- PROGRESS TOWARD HUMAN GENE THERAPY. Theodore Friedmann in *Science*, Vol. 244, No. 4910, pages 1275-1281; June 16, 1989.