of debilitating inherited human retinopathies termed RP. In the past few years, both linkage and candidate gene studies have been used to localize a number of disease-causing genes and, in some instances, identify specific mutations within candidate genes.

One can use prior knowledge of the biochemistry of visual transduction to predict genes that potentially may be involved in human retinopathies and hence direct future linkage and candidate gene studies. The genes encoding transducin, rhodopsin kinase, arrestin, and interstitial retinol binding protein are just a few of many potential candidates. It seems likely, for example, that the beta subunit of PDE, which has been shown to be responsible for the retinopathy observed in the *rd* mouse, may be involved in RP or in an RP-like retinopathy. Given the rapid techniques now available for such studies, it is probable that the majority of genes involved in RP will be characterized within the next few years. However, although great strides have been made toward identifying the genes responsible for RP, little as yet is known about the means by which the mutant proteins encoded by these genes cause photoreceptor cell death. Harnessing of expression systems to produce large quantities of biologically active proteins will enable investigation of the activities of normal and mutant proteins, such as rhodopsin and peripherin *RDS*, and may thereby shed light on pathogenic mechanisms in RP. Furthermore, the powerful technique of targeting potential retinopathy genes by homologous recombination will enable identification of more disease-causing genes and investigation of the course of the retinal degeneration observed in the chosen animal model. As research diversifies, we will not merely elucidate the molecular pathology of this group of distressing conditions but will also gain a greater understanding of the functions of these proteins in the healthy retina.

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Human Gene Therapy

W. French Anderson

Human gene therapy is a procedure that is being used in an attempt to treat genetic and other diseases. Eleven clinical protocols are under way at the present time, each with scientific and clinical objectives. Human genetic engineering raises unique safety, social, and ethical concerns.

Human gene therapy has progressed from speculation to reality in a short time. The first clinical gene transfer (albeit only a marker gene) in an approved protocol took place on 22 May 1989, almost exactly 3 years ago; the first federally approved gene therapy protocol, for correction of adenosine deaminase (ADA) deficiency, began on 14 September 1990. Now there are 11 active clinical protocols (Table 1) on three continents with nine more approved protocols about to begin and over a dozen additional protocols in various stages of development.

What are the objectives of these protocols? What are the scientific and clinical questions that they are asking? How safe are they? These are the questions that this review addresses. The extensive preclinical studies that support each clinical protocol are not covered.

An unsuccessful attempt was made in 1980 to carry out gene therapy for β-thalassemia with the use of calcium phosphate–mediated DNA transfer. Retroviral-mediated gene transfer was developed in the early 1980s in animal models (*1*). This technology is the principal procedure used today. Many recent reviews on retroviral vector development, packaging cell lines, and alternate gene delivery techniques (*2*) as well as on gene transfer and expression in cell cultures and in animal models (*3*) address the technical issues upon which human gene therapy relies.

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NeoR/TIL Gene Marking

The first federally approved human genetic engineering experiment, initiated in 1989, was for the transfer of gene-marked immune cells (specifically, tumor-infiltrating lymphocytes, or TIL) into patients with advanced cancer. The protocol had two primary objectives: (i) to demonstrate that an exogenous gene could be safely transferred into a patient and (ii) to demonstrate that the gene could be detected in cells taken back out of the patient (*4*).

The protocol asked a number of scientific and clinical questions, generated by several earlier experiments. In 1986, a clinical protocol for the treatment of advanced malignant melanoma with the newly dis-

covered class of immune cells called TIL was initiated at the National Institutes of Health (NIH) (5). These lymphocytes are T cells that are isolated directly from the tumor and that are then grown to large numbers in tissue culture in the presence of the T cell growth factor interleukin-2 (IL-2). After expansion in culture several thousand times, approximately 2×10^{11} TIL are given to the patient intravenously in addition to high doses of IL-2 in several days of treatment. Even in those patients who did not respond to all other therapy (including treatment with IL-2 alone), 35 to 40% of patients responded to this protocol (5).

The large-scale tissue culture and the large numbers of cells and IL-2 that are given to a patient make this procedure expensive and clinically difficult. Furthermore, 60 to 65% of patients fail to respond to this treatment, and even those who do respond will often fail after 6 to 12 months. It is likely that only a subset of the heterologous population of cells administered to a patient are effective in killing cancer cells in vivo; one goal of investigators is to determine which cells these are. It would be useful to be able to follow and study the administered cells in the patient's body to learn where they go and how long they survive. One approach would be to mark the TIL so that they could be tracked in the body. In^{111} has been used to follow TIL in patients, but In^{111} has a half-life of just 2.8 days, so that only short-term data could be obtained (6).

The NeoR/TIL clinical protocol proposed to take an aliquot of cells from TIL early in their culture, transfer into them a marker gene (the neomycin resistance gene NeoR obtained from *Escherichia coli*) with a retroviral vector (7), grow the marked cells in parallel with the unmarked cells, and then give both populations back to the patient. Periodic blood samplings would indicate how long the TIL survived in the bloodstream, periodic tumor biopsies would indicate if and roughly how many marked TIL were present in the tumors, and, if enough patients could be studied, perhaps a correlation could be drawn between the presence of marked TIL and the clinical response. It was also proposed to attempt to grow marked TIL out of tumors to determine if specific subsets of effective TIL could be identified.

The results from the first five patients have been published (8). Blood samples were taken before and during infusion, as well as 3 min, 1 hour, 24 hours, and on various days after infusion. Polymerase chain reaction (PCR) with NeoR probes (9) consistently detected marked TIL in the bloodstream from all patients for 21 days, from patient 3 on day 51, and from patient 5 on day 60. Patient 3 had a second TIL infusion on day 94, whereupon marked TIL were detected in the patient's blood samples up to day 189. The frequency of marked TIL was estimated to be 1 in 300 on day 3 in patient 5 (estimated with semiquantitative PCR), with values dropping to less than 1 in 10,000 cells in later samples. Marked TIL were detected in tumor biopsies taken from three of the five patients, including one taken on day 64 from patient 3. Thus, gene-marked TIL could consistently be detected (that is, more than one cell in 10^5 nucleated cells) in the bloodstream for 3 weeks, which is approximately the time span over which the patients received IL-2. Small numbers of gene-marked TIL were found in tumor samples as long as 9 weeks after infusion.

Table 1. Gene marker and therapy clinical protocols.

Protocol	Institute	Diseases	Date initiated
	Initiated: USA/RAC–approved		
Gene Marking			
NeoR/TIL	NIH, Bethesda, MD	Malignant melanoma	5/22/89
NeoR/bone marrow	St. Jude Children's Research Hospital, Memphis, TN	Pediatric AML	9/9/91
NeoR/bone marrow	St. Jude Children's Research Hospital, Memphis, TN	Neuroblastoma	1/16/92
NeoR/TIL	University of Pittsburgh, Pittsburgh, PA	Malignant melanoma	3/3/92
Gene Therapy			
ADA/peripheral blood T cells	NIH, Bethesda, MD	ADA-deficient SCID	9/14/90
TNF/TIL	NIH, Bethesda, MD	Malignant melanoma	1/29/91
TNF/tumor cells	NIH, Bethesda, MD	Advanced cancer	10/8/92
IL-2/tumor cells	NIH, Bethesda, MD	Advanced cancer	3/12/92
	Initiated: International		
Gene Marking			
NeoR/TIL	Centre Leon Berard, Lyon, France	Malignant melanoma	12/12/91
Gene Therapy			
Factor IX/autologous skin fibroblasts	Fudan University and Changhai Hospital, Shanghai, China	Hemophilia B	12/3/91
ADA/peripheral blood T cells + progenitor-enriched bone marrow cells	Scientific Institute, San Raffaele, Milan, Italy	ADA-deficient SCID	3/9/92
	Approved (uninitiated) protocols		
Gene Marking			
NeoR/bone marrow	University of Texas, M. D. Anderson Cancer Center, Houston, TX	CML	
NeoR/hepatocytes	Baylor College of Medicine, Houston, TX	Liver failure	
NeoR/bone marrow	Indiana University, Indianapolis, IN	Adult AML and ALL	
NeoR/TIL	University of California at Los Angeles, Los Angeles, CA	Malignant melanoma and renal cell cancer	
Gene Therapy			
LDL receptor/ hepatocytes	University of Michigan, Ann Arbor, MI	Familial hypercholesterolemia	
Herpes simplex thymidine kinase/ ovarian cancer cells	University of Rochester, Rochester, NY	Ovarian cancer	
HLA-B7/melanoma in vivo	University of Michigan, Ann Arbor, MI	Malignant melanoma	
Herpes simplex thymidine kinase/ cytotoxic T lymphocytes	Fred Hutchinson Cancer Research Center and the University of Washington, Seattle, WA	AIDS	
ADA/bone marrow	Institute for Applied Radiobiology and Immunology (TNO) with University of Leiden, Leiden, the Netherlands	ADA-deficient SCID	

An effort was made to culture NeoR-marked TIL isolated from posttreatment biopsies from one patient (whose cancer disappeared completely) (*10*). Mononuclear cells were isolated from an aliquot of a number of blood samples and placed in culture. After stimulation of the cells for several days with phytohemagglutinin (PHA), G418 at various concentrations was added to the different cultures. Only in samples taken during infusion and 1 hour after infusion was it possible to select out a population of G418-resistant TIL cells. In vitro studies demonstrated that when NeoR-TIL cells are mixed with standard TIL at a concentration of less than 1% and then selected in the presence of G418, the gene-marked TIL will not grow. Apparently, the toxic effects produced by the death of other TIL inhibits growth of NeoR-TIL, even when the G418 concentration starts low and is gradually or rapidly increased. Thus, once the NeoR-TIL are no longer in high concentration in the bloodstream (that is, 24 hours after infusion), their amount is too small to be selected out in vitro.

NeoR-marked TIL derived from tumor biopsies were also grown in G418 (*10*). Analysis of T cell receptor β-chain heterogeneity by Southern (DNA) blot was used to compare the infused TIL with the TIL obtained from the day 5 tumor biopsy sample. The TIL proliferating from the tumor had several subsets of bands in common with the infused TIL, but the population of TIL selected in G418 had only a few subsets in common with the infused TIL. These data could indicate that transduced TIL found in tumor biopsies were not a random assortment of infused TIL or, alternatively, that different subpopulations of TIL grow from the biopsies during the selection process. Thus, in this study it was not possible to correlate clinical effectiveness with subpopulations of marked TIL isolated from tumor biopsies. Because there were no side effects or pathology attributable to the gene transfer, the first objective of the protocol was established: it is possible to safely transfer a gene into a human patient. The identification of marked cells isolated from the bloodstream and from tumors validated the second objective.

Other gene marker protocols are being developed to investigate the characteristics of TIL therapy. Using a similar gene marker protocol, investigators at the University of Pittsburgh are studying whether TIL specifically "home" to tumor deposits. Their protocol uses semiquantitative PCR to compare the amount of marked TIL in tumor tissue with the amount measured in neighboring normal skin and muscle (*11*). To begin to identify specific subsets of TIL, investigators at the University of California at Los Angeles are labeling CD4 TIL with one retroviral vector and CD8 TIL with a second vector (*12*). The vectors are LNL6 (used in the NIH experiments) and G1N.40, a new vector with similar properties but which can be easily identified with different probes by PCR analysis (*13*). In addition, investigators at the Centre Leon Berard in Lyon, France, have initiated a TIL gene marker protocol similar to the one at NIH.

ADA Gene Therapy

There were two objectives of the ADA gene therapy protocol (*14*). The clinical objective was to evaluate the possible therapeutic efficacy of the administration of autologous lymphocytes transduced with a normal human ADA gene in an effort to reconstitute the function of the cellular and humoral immune system in patients with ADA-deficient severe combined immunodeficiency (SCID). The scientific objective was to evaluate in vivo survival of culture-expanded autologous T cells and the duration of expression of the inserted genes.

The rationale behind this protocol has a lengthy history. For many years, investigators had concentrated on β-thalassemia as the most likely initial candidate for gene therapy. However, it became clear by 1984 that diseases of hemoglobin synthesis were going to be particularly difficult to correct by gene therapy because of the complex regulation involved (hemoglobin is composed of two chains that are made on two different chromosomes but in exactly the same amounts—a sophistication of regulation that is still not fully understood). For gene therapy, it seemed wiser to initially focus on a genetic disease in which corrected cells might have a selective growth advantage in the patient (*15*). In other words, find a disease where the mutation was involved in DNA metabolism so that cell division was inhibited by the defect. Correction of the defect should produce normal cell division of the corrected cells against a background of very slow division by the uncorrected cells.

Three diseases fit this description on theoretical grounds: ADA deficiency, PNP (purine nucleoside phosphorylase) deficiency, and HGPRT (hypoxanthine-guanine phosphoribosyltransferase) deficiency (known as Lesch-Nyhan syndrome). The three enzymes that are defective in these diseases catalyze sequential steps in purine metabolism. Of these three diseases, ADA deficiency had been cured by matched bone marrow transplantation, the first performed 20 years ago. One year after the transplant, patients who were successfully treated often had all of their own blood cells except for their T cells, which were exclusively from the donor. Thus, ADA-normal T cells alone are curative and are able to overgrow the patient's own ADA-deficient T cells. For these reasons, ADA deficiency was chosen as the first disease for gene therapy (*15*), although originally the proposal was to use transduction of bone marrow cells rather than autologous T cells.

The plan for the ADA therapy protocol was to subject the patients to leukophoresis once a month, to isolate the mononuclear cells by Ficoll gradient, and to grow these cells in culture under conditions that stimulated T lymphocyte activation and growth (OKT3 antibody stimulation with growth in IL-2). Once the T cells were dividing, they would be incubated with the retroviral vector LASN (which carries a normal ADA gene as well as the NeoR gene) and then grown for only a few days before infusion into the patient.

The rationale for this protocol recognized that the proportional distribution of T cells that respond to different antigens in the normal T cell repertoire varies continuously depending on what antigens an individual is exposed to. Therefore, growing a large quantity of T cells by lengthy culture from a single sample (as in TIL immunotherapy) would increase the risk of oligoclonal predominance, potentially producing "holes" in the patient's T cell immune repertoire. Therefore, frequent small blood samplings followed by brief culture and subsequent infusion were used.

The protocol was initiated in September 1990. A 4-year-old-girl suffering from ADA deficiency received an intravenous infusion of her own gene-corrected T lymphocytes. She had been on enzyme replacement therapy, polyethylene glycol (PEG)–conjugated ADA, for 2 years, but after an initial improvement that lasted much of the first year, her T cell numbers decreased, she had frequent infections, and was anergic at the time the gene therapy treatment began. Over the next 10.5 months she received seven more infusions at 1- to 2-month intervals (together with the weekly PEG-ADA injections). Because of the improvement in her immune function studies and in her clinical condition as well as the presence of a significant number of gene-corrected T cells in her circulation (approximately 20 to 25%, on the basis of the amount of ADA in the mononuclear cell population and PCR analysis), she was followed without further gene therapy treatments for 6.5 months and then was begun on a program of maintenance infusions at 3- to 5-month intervals (*16*). A second patient, a 9-year-old girl, began treatment in January 1991 and has thus far received 11 infusions of gene-corrected autologous T cells (*16*). Both patients are maintained on weekly PEG-ADA injections.

The data from the protocol indicate that this therapy was clinically useful. Both patients have shown improvement in their clinical condition after gene therapy was begun as well as in a battery of in vitro and in vivo immune function studies (*16*). Both attend regular public schools and now have no more than the average number of infections. There have been no significant side effects from the cell infusions and no detected side effects from the presence of the transferred ADA gene itself. Data from patient 1 suggest that the ADA-deficient T cell population has a survival half-life in vivo of 30 to 35 days on the basis of the decline in circulating T cells when an infusion was missed early in the treatment procedure. PCR analysis and ADA enzyme activity in the mononuclear cell fraction suggest that the survival half-life of the ADA-corrected T cell population is much longer, perhaps three to five times longer (*16*). The T cell fraction, isolated from freshly drawn blood and tested for ADA expression, demonstrated a steady increase in the ADA level over the initial 10.5-month treatment period, starting under 1% of normal and reaching nearly 25% of normal after the eighth infusion (*16, 17*). This amount was maintained throughout the 6.5-month period without treatment. The assumption from these data is that the inserted ADA gene continues to be expressed in vivo for long periods of time, perhaps the lifetime of the T cell.

Even though both patients appear to be doing well, there is still a potential clinical concern. Because only mature T cells are being transduced, it is probable that holes are being left in their immune repertoires. In both children some of their immune function studies have not returned to normal, and positive skin tests are not observed for all antigens (*16*). To address this problem, the investigators propose an addition to the current protocol—to administer gene-corrected peripheral blood stem cells (that is, the CD34-enriched population) together with the gene-corrected mature T cells. A cell fraction highly enriched in hematopoietic progenitors and stem cells would be prepared by immunoselection with a CD34-specific antibody (*18*) and transduced with an ADA vector under culture conditions designed to facilitate retroviral-mediated gene transfer with preservation of repopulating stem cell activity (*19*). The two cell populations would be transduced with different ADA vectors (LASN, the vector presently being used, and G1NaSvAd, a new vector that can be differentiated from LASN by PCR probes). Thus, it should be possible to determine the contribution of CD34-enriched cells in comparison to that of mature T cells. Recent evidence in primates suggests that, under certain experimental conditions, it may be possible to transduce totipotent stem cells taken from the bone marrow by retroviral-mediated gene transfer (*20*).

There are three scientific questions asked by the protocol addition. (i) Can one detect gene-marked lymphoid and myeloid blood cells that were derived from the peripheral blood CD34-enriched cell population? If so, this would suggest that prelymphoid as well as premyeloid progenitor cells are in the CD34-enriched population and that they can be transduced with retroviral vectors. (ii) Will it be possible to determine (by means of specific insertion sites detected by Southern blots of cloned cells) whether or not there are lymphoid and myeloid cells that came from the same transduced precursor cell? If so, this would be evidence for stem cell gene transfer. (iii) How long can gene-marked precursor cells be detected in the circulation? If stem cells are transduced, the answer may be the lifetime of the patient.

The clinical questions being asked by the protocol addition are: (i) Will the clinical effects from the administration of the gene-corrected CD34-enriched progenitor cell population last longer than the effects from the administration of the gene-corrected mature T cell population? and (ii) will the immune protection afforded by gene-corrected CD34-enriched cells be broader (that is, will pre–T cells be corrected so that new specificities can be added to the immune repertoire as needed)? This protocol modification has received NIH Recombinant DNA Advisory Committee (RAC) approval and is awaiting Food and Drug Administration (FDA) approval.

Investigators in Milan, Italy, have just begun a gene therapy protocol for ADA deficiency similar to the proposed addition described above. The rationale and questions asked are essentially identical. In the Milan protocol, ADA gene–corrected peripheral blood T cells (stimulated in culture with PHA, OKT3 antibody, and IL-2) were given to a 5-year-old boy in March 1992. In April, a mixture of ADA gene–corrected T cells and gene-corrected progenitor-enriched bone marrow cells (containing a vector that can be differentiated from the T cell vector) were given to the same patient (*21*). In addition, on the basis of their encouraging results in rhesus monkeys (*20*), investigators in the Netherlands have received approval for a bone marrow gene therapy protocol for ADA deficiency.

Cancer Gene Therapy

The first cancer gene therapy protocol was a direct outgrowth from the NeoR/TIL gene marker protocol. After it was shown that gene-modified TIL could be safely given to patients, a new protocol was initiated in which the gene for tumor necrosis factor (TNF) was added to the vector (*22*). The objective was to make the TIL more effective against advanced malignant melanoma.

TNF itself is a powerful anticancer agent in mice. In humans, however, its toxic effects become profound at 8 μg per kilogram of body weight, whereas the effective dose in mice is 400 μg/kg (mice can tolerate the high dose) (*23*). By putting a TNF gene into TIL, and then letting the TIL "home" to tumor deposits, it may be possible to develop effectively high doses of TNF in tumor sites and avoid systemic side effects. However, because the bulk of TIL cells are trapped (and probably destroyed) in liver, spleen, and lungs, and the production of TNF from the exogenous gene is from a heterologous promoter, production of large amounts of ectopic TNF with toxic effects is possible. Thus, a Phase I safety trial was needed to determine if toxic concentrations of TNF might develop in the liver or other organs.

The first patient began treatment in January 1991 and a number of patients are currently under treatment. There have been no side effects yet from the gene transfer and no apparent organ toxicity from secreted TNF, but it is still too early in the protocol to determine if the procedure is effective as a cancer therapy.

There are at least two ways to improve TIL immunotherapy by gene transfer: either add a gene to the TIL or tumor-specific T cells to make them more effective (as discussed above) or add a gene to the tumor cells in order to induce the body's immune system to make more effective TIL. Two protocols have begun in which either the TNF gene or the IL-2 gene is inserted into tumor cells that were isolated from a patient and grown in culture (*24*). These approaches are based on animal studies that have shown that immunization with tumor cells transduced with certain cytokine genes produces systemic antitumor immunity mediated by T cells. In these protocols, the cytokine-secreting autologous tumor cells are then injected subcutaneously and intradermally into the patient's upper thigh. Twenty-one days later the injection site and draining lymph nodes are removed, placed in culture, and grown under conditions to encourage T cell growth. The expanded lymph node T cells are given to the patient with IL-2. Data from these "tumor vaccine" studies should be available later in the year.

Two other cancer gene therapy protocols have been approved by RAC and are awaiting FDA approval. These are the studies from the University of Michigan and from the University of Rochester (Table 1).

Other Protocols

Gene therapy. Two additional gene therapy protocols have been approved but have not yet begun. The first, from the University of Michigan, proposes to insert a low density lipoprotein (LDL) receptor gene into hepatocytes obtained from patients suffering from familial hypercholesterolemia (a result of a defective LDL receptor gene) (*25*). The gene-corrected hepatocytes would be injected back into the portal circulation of the patient. The second, from the University of Washington, is really a cell therapy for a complication of acquired immunodeficiency syndrome (AIDS) in which a suicide gene is inserted into the therapeutic cytotoxic T cells to afford protection in case the T cells become too toxic (*26*). Finally, a gene therapy protocol has been initiated in China for hemophilia B. A retroviral vector containing a factor IX gene has been used to transduce autologous skin fibroblasts growing in culture. The factor IX–secreting autologous fibroblasts were then injected subcutaneously into the patients. The protocol was begun in December 1991 on two patients; results are not yet available.

Gene marking. Two types of gene marker protocols, in addition to those involving TIL, have been approved. The first category of protocol is for using gene marking to study bone marrow reconstitution and the mechanism of relapse in autologous bone marrow transplantation in patients with leukemia or neuroblastoma. Under way since September 1991 at St. Jude Children's Research Hospital, a study of children with acute myelogenous leukemia (AML) in first remission (*27*) is attempting to understand what happens when an AML patient who has received back his or her own purged bone marrow has a relapse. Does the relapse arise from residual cells in the transplanted marrow that were not killed by the purging techniques or does it arise from residual systemic disease in the patient that was not eradicated by the ablative therapy?

This question is of considerable clinical importance because, if the leukemic cells do come from the marrow implant, then harsher purging techniques would be necessary. Such techniques damage the healthy marrow cells, however, thereby preventing or delaying reconstitution in some cases. If relapse arises from cells in the patient's body, then more strenuous ablation would be appropriate (with its toxic side effects), but the marrow transplant could be treated more gently. Because only the purged marrow would be gene-marked, a relapse composed of gene-marked cells would indicate that the marrow transplant was responsible for, or at least contributed to, the relapse. If marked tumor cells were found during relapse, the technique may also allow analysis of the efficiency of different purging techniques. The difficulty is that only some of the cells in the marrow will be gene-marked; therefore, the failure to detect gene-marked cells in a relapse would provide no useful information. Data from the first relapsed patient are now available, and gene-marked cells have been identified (*28*).

Also under way at St. Jude Children's Research Hospital is a similar marking study for pediatric patients with neuroblastoma (*29*). Marker protocols have been approved for the study of adult chronic myelogenous leukemia (CML) at the M. D. Anderson Cancer Center (*30*) and of adult AML and acute lymphocytic leukemia (ALL) at Indiana University (*31*).

The second category of gene marker protocol is where marking is to be used to study hepatocellular transplantation in children with acute liver failure with no other medical or surgical options. The protocol, from Baylor College of Medicine, proposes to isolate heterologous hepatocytes from a donor liver, insert a marker NeoR gene by means of a retroviral vector, and inject the donor hepatocytes (some of them marked) into the diseased liver in order to provide essential hepatic functions (*32*). This protocol is scheduled to begin shortly.

Safety Considerations

How safe are these protocols? The safety considerations in retroviral-mediated gene transfer and therapy clinical protocols have been extensively reviewed (*33*). There is now experience from the equivalent of 106 monkey-years and 23 patient-years in individuals who have undergone retroviral-mediated gene transfer. Side effects from the gene transfer have not been observed, pathology as a result of gene transfer has not been found, and there has never been a malignancy observed as a result of a replication-defective retroviral vector.

Recently, however, investigators at NIH described three monkeys who developed malignant T cell lymphomas after a bone marrow transplantation and gene transfer protocol with a helper virus–contaminated retroviral vector preparation (*34*). The helper virus was probably directly responsible for these lymphomas. This finding strongly reaffirms the necessity for clinical protocols to use helper virus–free vector preparations, as is required for all protocols approved by RAC and by FDA.

Ethical and Social Implications

The ethical and social implications of somatic cell gene therapy have been discussed (*35*), and there is now a general consensus that somatic cell gene therapy for the purpose of treating a serious disease is an ethical therapeutic option. However, considerable controversy exists as to whether or not germline gene therapy would be ethical (*36*). The issues are both medical and philosophical.

The medical concern is that genetic manipulation of the germline could produce damage in future generations. Medicine is an inexact science; we still understand very little about how the human body works. Well-intentioned efforts at treatment with standard therapeutics can produce unexpected problems months or years later. Altering the genetic information in a patient's cells may result in long-term side effects that are unpredictable at present. Until the time comes that it is possible to correct the defective gene itself by homologous recombination (rather than just inserting a normal copy of the gene elsewhere in the genome), the danger exists of producing a germline mutagenic event when the "normal" gene is inserted. Therefore, considerable experience with germline manipulation in animals, as well as with somatic cell gene therapy in humans, should be obtained before considering human germline therapy.

Besides the medical arguments, there are a number of philosophical, ethical, and theological concerns. For instance, do infants have the right to inherit an unmanipulated genome, does the concept of informed consent have any validity for patients who do not yet exist, and at what point do we cross the line into "playing God"? The feeling of many observers is that germline gene therapy should not be considered until much more is learned from somatic cell gene therapy, until animal studies demonstrate the safety and reliability of any proposed procedure, and until the public has been educated as to the implications of the procedure. The NIH RAC plans to initiate the public debate.

There is also considerable concern about using gene transfer to insert genes into humans for the purposes of enhancement—that is, to try to "improve" desired characteristics (*37*). The same medical issues arise: we have too little understanding of what normal function is to attempt to improve on what we think is "normal." Correction of a genetic defect that causes serious illness is one thing, but to try to alter a characteristic such as size (by administration of a growth hormone gene to a normal child, for example) is quite another. This area is further clouded by major social implications (*37*) as well as by the problem of how to define when a given gene is being used for treatment (or for preventing disease) and when it is being used for "enhancement."

The Future

How much impact will gene therapy have on medical practice in the future? Not a great deal so long as the technique is carried out as it is today, where cells are removed from a patient, the desired gene is inserted, and the gene-corrected cells are returned to the patient. This procedure is too dependent on specialized technologies, is too expensive, and requires too much scientific and medical expertise to be used extensively except in major medical centers. The many clever applications of gene transfer that investigators are devising ensure that gene therapy will be applied to a broad range of diseases over the next several years, but only thousands, not millions, of patients are treatable by current techniques.

Gene therapy will have a major impact on the health care of our population only when vectors are developed that can safely and efficiently be injected directly into patients as drugs like insulin are now. Vectors need to be engineered that will target specific cell types, insert their genetic information into a safe site in the genome, and be regulated by normal physiological signals. When efficient vectors of this type are produced—retroviral, viral, synthetic, or a combination of all three—then gene therapy will probably have a profound impact on the practice of medicine. As information from the Human Genome Project becomes available concerning the entire library of genetic information in our cells, then gene therapy will probably be used not only to cure an array of diseases, but also to prevent many disorders by providing protective genes before the diseases become manifest. Although the medical potential is bright, the possibility for misuse of genetic engineering technology looms large, so society must ensure that gene therapy is used only for the treatment of disease.

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