[Frontiers in Bioscience, 4, d468-475, May 15, 1999]

GENE THERAPY FOR THE TREATMENT OF AIDS: ANIMAL MODELS AND HUMAN CLINICAL EXPERIENCE

Rafael G. Amado[1], Ronald T. Mitsuyasu[1] and Jerome A. Zack[2]

[1]Division of Hematology/Oncology, [2] and Department of Microbiology & Molecular Genetics, UCLA School of Medicine, 11-964 Factor Building, Los Angeles, CA 90095

TABLE OF CONTENTS

1. ABSTRACT

Although antiretroviral drug therapy has had a significant impact on the natural history of HIV infection, complete virus eradication still remains an unattainable goal. Drug-mediated virological control only occurs transiently, in part as a result of the development of drug resistance. Gene therapy for the treatment of AIDS is a promising area of research that has as its goal the replacement of the HIV-infected cellular pool with cells engineered to resist virus replication. A variety of anti-HIV genes have been designed and tested in laboratory systems, and available results from pilot clinical trials demonstrate the safety and feasibility of this approach. Obstacles to effective application of this technology include partial protection of HIV resistance genes, lack of effective vectoring systems, and unregulated gene expression. Herein, we review recent advances in transduction methods, data from *in vivo* preclinical studies in relevant animal models, and emerging results derived from pilot clinical gene therapy studies.

2. INTRODUCTION

In recent years, enormous advances have been made in our understanding of HIV pathogenesis and in the development of effective therapeutic strategies to combat HIV infection. Combination antiretroviral therapeutic agents containing protease and reverse transcriptase inhibitors are capable of reducing HIV RNA in peripheral blood to undetectable levels (1). Several groups have reported that HIV replication can also be decreased in lymphoid tissue through combination therapy (2). The successful use of drug combinations has raised expectations for the possibility of complete eradication of the virus from infected individuals. However, the retroviral life cycle presents unprecedented therapeutic challenges to achieving this goal, as cell-associated virus can act as a reservoir of HIV infection in quiescent cells, and remain protected from the immune system and the effects of drug therapy (3, 4). The high burden of cell-associated virus, the long survival period of resting infected cells, and the ability of these cells to be induced to produce virus are barriers against achieving a cure for infected patients. Indeed, cessation of antiretroviral therapy in treated individuals has thus far uniformly resulted in re-activation of HIV replication. Other limitations of drug therapy include toxicity, cumbersome schedules that hinder compliance and the development of drug resistance.

The use of cell and gene therapy strategies to combat HIV infection remains a promising approach in the fight against AIDS. Certain genetic disorders and infectious diseases such as AIDS may benefit from the introduction of therapeutic genes into a small fraction of hematopoietic stem cells (HSC). Stable introduction of therapeutic anti-HIV genes into HSC has the potential to reconstitute the immune system with protected cells in infected individuals. The progeny of these cells could potentially exhibit resistance to HIV infection, and could therefore have a selective survival advantage and become the predominant clones contributing to hematopoiesis. Such accumulation of cells bearing the transferred gene has been described in a gene therapy clinical trial involving pediatric patients with severe combined immunodeficiency (5).

At present, the following significant hurdles hinder our efforts to effectively apply gene therapy principles to the clinic: a) limited protection - although a number of gene therapy strategies aimed at preventing HIV replication have been designed (such as antisense oligonucleotides, ribozymes, decoy regulatory RRE or TAR sequences, transdominant Rev or Tat proteins, mutant

tRNA molecules, intracellular antibodies and chimeric T-cell receptors), they are of limited efficacy when infections are performed *in vitro* with multiplicities of infection (m.o.i.) above a certain low threshold; b) limited transduction efficiency - because HIV infects cells of different hematopoietic lineages, genetic therapies for the treatment of AIDS presumably would require the therapeutic gene to be stably introduced into a chromosome of an early hematopoietic progenitor with multilineage potential. Retroviral vectors derived from the Moloney murine leukemia virus (MMLV) have been used extensively to transduce $CD34^+$ cells. However, these vectors can only achieve integration when the target cell is actively progressing through the cell cycle (6). Induction of stem cells to divide during the transduction process leads to stem cell commitment and loss of long-term reconstitution potential. Human studies employing murine vectors to transduce $CD34^+$ cells have revealed poor efficiency of gene marking in mature hematopoietic cells, which is often short-lived as a result of transduction into committed hematopoietic progenitors (7); c) limited expression - expression of therapeutic genes driven by viral promoters is often suboptimal, and is not regulated or tissue-specific.

This review will describe alternatives currently being developed to overcome some of the obstacles mentioned above. In addition, we will review the use of the SCID-hu mouse system as a model of gene therapy for AIDS. Lastly, we will outline some of the current clinical experience available to date in the field of T-cell and stem cell gene therapy for HIV.

3. OPTIMIZATION OF GENE TRANSFER TECHNOLOGY

A number of strategies are being investigated in an effort to increase transduction efficiencies of hematopoietic progenitors without altering the stem cell potential of these cells. Hanenberg et al. reported that fibronectin, an abundant protein present in the bone marrow microenviroment, can facilitate retroviral transduction by co-localizing vector and target cells (8). A fragment of fibronectin termed CH-296, which encompasses binding sites for integrins VLA-4 and VLA-5, as well as a heparin-binding domain, has been shown to greatly facilitate gene transfer into hematopoietic precursors, as assayed by gene presence in colonies (colony-forming units, CFU) obtained in semi-solid medium, and into primary T-cells (9, 10). Cytokines known to mediate effects on early precursors such as megakaryocyte growth and development factor (MGDF) (11), stem cell factor (SCF) or FLT-3L can enhance gene transfer with murine vectors, while preserving engraftment potential. Transductions performed during ex vivo stem cell expansion may result in a graft containing high numbers of transduced precursors. Novel packaging cell lines derived from human lines can produce high-titer vector in the absence of replication-competent retrovirus. In animal models, myelosuppression prior to infusion of the transduced stem cell product has been associated with enhanced and longer-term gene presence in mature cells (12). A recent clinical study employing the multidrug resistance (mdr) gene, carried out in the context of autologous progenitor cell transplantations for the treatment of refractory germ cell tumors, has demonstrated a median level of expression of vector-encoded mdr-positive CFU of 12% at 6 months following $CD34^+$ cell infusion (13). Similarly, the advent of efficient methods for mobilization and peripheral blood stem cell procurement has made possible the use of repeated infusions of very high numbers of transduced cells. In a study employing the $gp91^{phox}$ gene in patients with chronic granulomatous disease, such an approach has been associated with levels of transgene expression in neutrophils of 1/20,000. Patients in this study received up to 4.4×10^7 cells/kg with each of two tandem treatments, without myeloablation (14). The levels of gene transfer achieved with these strategies may be relevant in the HIV setting, and may have an impact on the disease course, particularly if the genetic treatments are performed in conjunction with adjuvant pharmacological or immune therapies.

An area of intense research in the field of gene therapy is the development of efficient retroviral systems derived from lentiviruses. Lentiviral vectors hold great promise for gene therapy applications and, in particular, for the treatment of HIV infection, as they can mediate gene transfer into quiescent cells. Although published reports suggest that lentiviruses cannot efficiently transduce truly quiescent cells (cells in the G_0 state) (15), unlike murine vectors, HIV-based vectors can achieve effective and sustained transduction and expression in non-dividing cells such as neurons, retinal epithelial cells, myocytes and unstimulated $CD34^+$ hematopoietic cells (16-20), as the pre-integration complex of HIV (matrix, Vpr and integrase) has karyophilic properties. Recently, Uchida and colleagues demonstrated that lentiviral but not murine retroviral vectors could mediate gene transfer into hematopoietic cells selected for a primitive stem cell phenotype (19), and Miyoshi et al have reported that CD34+ cells transduced with an HIV based lentiviral vector can repopulate non-obese diabetic/severe combined immunodeficient (NOD/SCID) mice long-term (20).

Another potential advantage of lentivectors in the setting of HIV infection is their potential for mobilization by wild-type virus, which supplies all essential gene products for packaging and infection. Such a phenomenon would be associated with high levels of transduction in the very cells that require protection from wild-type HIV. In addition, lentivectors have the potential to mediate tissue-specific gene expression, as tat-inducible vectors, in which genes are driven by the HIV LTR promoter, are only transcriptionally active in cells that are expressing tat from wild-type HIV. Recent data also suggest that HIV-based vectors may confer protection against wild-type HIV, even in the absence of a custom-designed anti-HIV gene. This effect may be mediated by competition for packaging or cellular factors (21).

4. THE SCID-HU MOUSE AS A MODEL FOR HIV GENE THERAPY

The SCID-hu mouse is generated by transplantation of human fetal liver and thymus fragments

under the kidney capsule of the SCID mouse, resulting in the growth of a conjoint thymus/liver ("Thy/Liv") organ that allows development of human thymocytes for periods up to one year (22, 23). After maturation, these implants histologically and phenotypically resemble the normal human thymus, and can be infected by HIV-1. In addition to serving as a model for HIV infection, the SCID-hu mouse can be used to study thymopoiesis and hematopoiesis, as the Thy/Liv organ contains precursors capable of undergoing differentiation into T-lymphoid, myeloid and erythroid lineages. We have used this model to ascertain whether *de novo* T-cell and hematopoietic stem cell differentiation can occur after the supporting thymic microenviroment has been exposed to large amounts of HIV. These are fundamental requisites for the successful implementation of HIV stem cell gene therapy.

4.1. Effects of HIV infection on the thymic microenviroment

We investigated whether HIV infection interferes with the ability of thymic stromal elements to support thymopoiesis. Six weeks following HIV infection, implants display depletion of virtually all CD4/CD8 double-positive and most CD4 single-positive cells. Pre-treatment of animals with the triple-drug combination, AZT, ddI and a protease inhibitor, prevents infection, as evidenced by inhibition of CD4 depletion and absence of HIV proviral DNA in thymocytes. Institution of triple-drug combination therapy 6 weeks post-infection allows a transient resurgence of endogenous thymopoiesis, as evidenced by an enhanced CD4/CD8 profile at 11 weeks post-infection (24). Drug treatment decreases circulating viral load one week following drug administration. However, viremia can be detected in some animals one month after initiation of therapy, suggesting that breakthrough virus replication is responsible for the eventual decrease in $CD4^+$ cells following an initial recovery. Analysis of proviral protease gene sequences obtained from infected animals did not reveal substitutions previously associated with resistance to protease inhibitors, suggesting that selection for protease inhibitor-resistant strains is not responsible for the eventual decline of $CD4^+$ thymocytes. To further study the mechanism for this secondary thymocyte decline observed after drug therapy, we infected human thymic implants with a reporter strain of HIV that expresses the murine heat stable antigen (HSA) on the surface of infected cells (25). Detailed kinetics of T-cell depletion and viral expression revealed that the eventual decline of $CD4^+$ thymocytes observed in this system in the presence of continuing antiretroviral therapy is associated with a rapid increase in the fraction of these cells that express HIV. These results suggest that viral breakthrough is at least partially responsible for the temporary nature of drug-induced T-cell resurgence observed in this system, and supports the testing of strategies such as gene therapy in conjunction with pharmacological approaches to provide additional protection that may prevent viral breakthrough in HIV-positive patients.

We also used the SCID-hu system to determine whether T-cell differentiation from exogenous progenitors could take place in the setting of virus replication and following drug therapy. Implants were injected with HIV, and CD4 depletion was verified by flow cytometry 4 weeks later. Subsequently, animals were started on anti-retroviral treatment and were injected with exogenous HLA-A2-disparate $CD34^+$ progenitors. Biopsies performed 3 weeks later were analyzed by three-color flow cytometry for expression of HLA-A2, CD4 and CD8. $CD4^+/CD8^+$ and $CD4^+/CD8^-$ cells from both HLA-$A2^+$ and HLA-$A2^-$ phenotypes were significantly increased 1 month after administration of antiretroviral therapy, as compared to untreated implants ($p=0.0001$). Only donor CD8 but not $CD4^+$ cells were seen in control implants that did not receive drug treatment, suggesting that although donor-derived $CD4^+$ thymocytes were depleted as a result of ongoing virus replication, HIV infection did not preclude thymocyte differentiation from exogenous progenitors (24). Taken together, these results indicate that HIV-infected thymic implants can support renewed thymopoiesis, and that the HIV-exposed thymic stroma remains functionally active. These results support the implementation of cellular and gene therapeutic strategies for the treatment of AIDS.

4.2. Effects of HIV-1 infection on hematopoiesis

As HIV infects myeloid elements, it is important to ascertain whether myeloid differentiation can occur in the setting of viral replication, and whether drug therapy alters the kinetics of progenitor cell differentiation. Hematopoietic progenitors derived from fetal liver tissue are present in SCID-hu implants, and can give rise to myeloid and erythroid colonies in semi-solid medium. We and others have determined that 3 weeks following HIV infection, the number of colonies obtained from progenitors in SCID-hu implants is significantly reduced (26, 27). When equal numbers of cells from uninfected and infected implants were mixed and plated on methylcellulose, the ability of uninfected precursors to form colonies was not suppressed, indicating that the inhibition of colony formation in precursors from infected implants was not due to HIV spread *in vitro*. Proviral DNA was detected in only 10% of the colonies at low levels (<0.05 copies/cell), indicating that infection of the precursor that gave rise to the clonal population did not occur.

To determine whether inhibition of colony formation was the result of alterations of the microenviroment or of hematopoietic precursors, we administered triple-combination antiretroviral therapy to animals following HIV-induced progenitor cell depletion at 3 weeks post-infection. Up to a 35 and 70% resurgence of colony-forming units was observed for erythroid and myeloid colonies respectively 7 weeks post-infection, demonstrating that the inhibitory effects of HIV on hematopoiesis were reversible (26). These results parallel those observed for thymopoiesis resurgence, and suggest that the microenviroment is preserved and capable of supporting hematopoiesis if viral replication is effectively suppressed.

4.3. Stem cell gene therapy studies in the SCID-hu system

Stable gene delivery into mature components of the immune system requires introduction of the desired

genes into precursor cells that are capable of stably reconstituting hematopoiesis. Retroviral vectors are widely used for gene delivery both in the laboratory and in clinical applications. Although significant transduction rates of hematopoietic progenitors can be achieved *in vitro*, these rates do not predict the efficiency of reconstitution with differentiated cells containing the transgene *in vivo*. Therefore, the study of novel methods to improve transduction efficiency, expression and efficacy of therapeutic genes requires the use of a transplantation model.

$CD34^+$ cell transplant studies performed in the SCID-hu model have demonstrated that exogenous precursors injected into previously irradiated implants can reconstitute normal thymopoiesis. Retroviral marking of T-cell progenitors allows reconstitution of implants with thymocytes harboring the transgene. As few as 5×10^4 $CD34^+$ cells can engraft and reconstitute thymic implants. The transgene (neo^R) is expressed at low levels in human thymocytes without any apparent effects on thymocyte differentiation (28). Using highly concentrated MMLV retroviral stocks pseudotyped with the vesicular stomatitis virus G-protein (VSV-G), retroviral transduction of $CD34^+$ cells can be achieved at high levels (29). In one study, thymocytes derived from transduced $CD34^+$ cells contained the transgene at levels of approximately 1 copy per cell. Expression of a vector containing the firefly luciferase (*luc*) gene in thymocytes was present in 11/13 reconstituted Thy/liv implants, and ranged from 485 to 5,806 relative light units ($RLU/10^5$ total thymocytes). The calculated levels of *luc* expression were much lower than those seen in input $CD34^+$ cells or those achieved in *ex vivo* experiments, suggesting that loss of expression of MMLV vectors occurs with T-cell differentiation.

To enrich for progenitor cells expressing the transgene, a retroviral vector was used that contains the reporter gene, Thy1.2, which was shown previously by flow cytometry to be effective for monitoring gene expression in individual cells. Injection of unsorted $CD34^+$ cells transduced with high-titer VSV-G-pseudotyped vector results in levels of Thy1.2 expression in mature thymocytes of less than 3%. To enrich exogenous progenitors for cells expressing the transgene, transduced $CD34^+$ cells were purified by flow cytometry for co-expression of both CD34 and the Thy1.2 marker, and injected into irradiated SCID-hu Thy/Liv implants. Thymocytes were analyzed 4 to 6 weeks later for donor reconstitution and for the presence and expression of the transgene. After reconstitution with exogenous cells, the SCID-hu mouse becomes a mosaic of both donor and endogenous cells. Thymocytes from exogenous progenitors were identified by Y chromosome PCR, using progenitors selected from male tissue and injected in a background of a female implant. Engraftment was estimated, using quantitative PCR for Y chromosome versus β-globin sequences (as an indicator of total DNA), and was shown to vary between 18% and 75%. The level of vector DNA ranged from 0.1 to 8.5 copies of Thy1.2. per donor-derived thymocyte. When normalized by the percentage of donor cells, Thy1.2 expression could be detected in up to 70% of mature donor thymocytes (30). In spite of immunoselection, a proportion of thymocytes bearing Thy1.2 DNA failed to express the transgene. Thymocytes sorted into Thy1.2-positive or -negative subfractions were analyzed by PCR for the presence of Thy1.2 DNA. Although Thy1.2 DNA was present in both fractions, the Thy1.2-expressing fraction contained multiple gene copies per cell. These results indicate that gene dosage may be important for efficient expression of transduced cells. This research underscores the need for testing stem cell gene therapy strategies in an *in vivo* model.

Attempts to model the efficacy of gene therapy reagents in the SCID-hu system have been limited by the relatively low level of presence of vector in mature thymocytes. This limitation is secondary to limited transduction efficiency, and to the resurgence of endogenous thymocytes that dilutes the transduced cell pool. In addition, expression of these vectors is suboptimal, and as suggested above, may require multiple integration copies. Lastly, large amounts of virus are produced in this system, which may overwhelm the protective properties of these genes. Bonyhadi and colleagues studied whether progenitor cells transduced with an antiviral gene and induced to differentiate into T-cells in the SCID-hu system could display resistance to HIV replication. Mobilized peripheral blood progenitor cells were transduced with a bicistronic retroviral vector that encodes the RevM10 and the Lyt-2α (murine CD8α) genes, followed by immunoselection for CD8 to allow enhanced engraftment in SCID-hu implants. Following thymocyte differentiation, Thy/Liv implants were stimulated *ex vivo*, purified by flow cytometry for donor HLA, CD4 and Lyt2 expression, and challenged with 200-1000 $TCID_{50}$ of various HIV isolates. Viral replication was reduced by 1 to 2 log orders over values observed with similarly sorted cells bearing a mutated RevM10 gene (31). Although sorting allowed for enrichment of cells bearing the protective gene and gene expression was enhanced by *in vitro* culture, this experiment established that T-cells derived *in vivo* from RevM10-transduced progenitors could inhibit viral replication.

5. T-CELL GENE THERAPY FOR SIV

Recently, Donahue and colleagues reported that they had genetically engineered $CD4^+$ cells to partially prevent SIV replication *in vivo* (32). $CD4^+$ cells were selected from peripheral blood mononuclear cells (PBMC) obtained from 6 rhesus macaques. Transduction was performed in 3 animals with a vector containing an antisense to *tat/rev* sequences, by repeated *ex vivo* rounds of gene transfer that resulted in 30-40% gene transfer efficiency. Cells were infused after a 3-day expansion period. Three animals infused with untransduced cells served as controls in this study. Following cell infusion, all 6 animals were inoculated with SIV. At the time of infection, the levels of $CD4^+$ cells bearing the therapeutic gene ranged from 2.4% to 9.6%. These levels declined to 0.2-1% after approximately 10 weeks, and remained constant for over 36 weeks. Transgene expression was demonstrated at 27 weeks without *in vitro* stimulation. All

6 animals became infected; however, a significant reduction in viral load, as evidenced by a decrease in circulating p27, as well as virus production from PBMC ($p<0.1$), was documented in animals receiving gene-modified cells. In sharp contrast to control animals, no loss in $CD4^+$ cell numbers were observed in the animals that received autologous transduced lymphocytes ($CD4^+$ T-cell numbers in these animals were similar to those of uninfected controls up to 175 days post-inoculation). Lymph node tissue sampled 1 month after treatment and subjected to *in situ* hybridization demonstrated less pathology (follicular hyperplasia, paracortical expansion and lymphoid depletion) and lower levels of viral RNA for animals receiving autologous genetically modified $CD4^+$ cells as compared to control animals. Although this procedure did not prevent infection or block disease progression completely, it significantly attenuated the initial phase of infection by inhibiting viral replication in a relevant animal model of HIV infection.

6. HUMAN CLINICAL EXPERIENCE WITH THE REV TRANSDOMINANT PROTEIN, REVM10

Rev is a 116-amino acid viral protein essential for viral replication. The *rev* gene is encoded by a fully spliced mRNA expressed early in viral infection. Rev shuttles between the nucleus and the cytoplasm, and acts post-transcriptionally to mediate cytoplasmic export of unspliced (≈9 kb) and singly spliced (≈4 kb) viral RNA. This function requires direct and highly specific Rev binding to the Rev-response element (RRE). Low levels of Rev are associated with latent infection (33). Transdominant Rev proteins have been successfully utilized to inhibit viral replication *in vitro*. RevM10 is a protein derived from Rev by the introduction of mutations into a highly conserved leucine-rich domain that interacts with cellular factors. These mutations result in substitutions of aspartic acid for leucine at position 78, and of leucine for glutamic acid at position 79. As a result, RevM10 competes with wild-type Rev for binding to the viral RRE without eliciting Rev function. RevM10 has been widely modeled in pre-clinical experiments, and has been shown to effectively inhibit HIV replication. Cell lines stably transfected with RevM10 have been shown to inhibit HIV replication. Introduction of RevM10 by viral and non-viral methods into primary T-cells *in vitro* resulted in significant inhibition of viral replication (34) and, as described above, SCID-hu thymocytes derived from hematopoietic stem cells transduced with RevM10 are resistant to HIV infection after expansion *in vitro* (31).

The HIV inhibitory effects of RevM10 have been studied in two human clinical studies involving *ex vivo* introduction of RevM10 into $CD4^+$ T-cells, followed by expansion and re-infusion into 3 HIV-infected patients with CD4 counts of 400-500 cells/mm^3. As a surrogate marker for RevM10 protection against HIV replication, survival of $CD4^+$ cells bearing the RevM10 gene was studied over time and compared to that of $CD4^+$ cells bearing a mutated RevM10 gene. In the first published study of Woffendin et al. (35), the investigators utilized plasmids encoding the neo^R gene and either RevM10 or ΔRevM10, an identical plasmid containing an internal 13-bp deletion. The genes were introduced into CD8-depleted peripheral blood leukocytes (PBL) by gold particle-mediated gene transfer. Equal numbers of cells were transfected separately, then mixed in equal proportions and simultaneously re-infused into the patients. The antiviral effects of RevM10 were demonstrated *in vitro* in transfected G418-selected cells by measuring p24 and reverse transcription (RT) levels after challenge with HIV at low MOIs (0.005 to 0.05). RT analysis revealed a 4-5.5-fold reduction in RT activity at day 7 post-infusion. The persistence of gene-bearing cells was determined by limiting dilution PCR in PBL. No gene signals were detected when patients received 10^9 cells. Two patients were subsequently infused with 2×10^{10} cells transfected at ≈10% efficiency. Both RevM10 and ΔRevM10 genes were detectable at equal frequencies ($>10^5$) 1 hour post-infusion. A 10-fold increase in the relative ratio of RevM10- over ΔRevM10-transduced cells was observed after 1 week. The longest persistence of RevM10-transduced cells was 8 weeks in 1 patient, at frequencies of $1/10^4$ at 2 weeks, $1/10^5$ at 4 weeks and $1/10^6$ at 8 weeks. No ΔRevM10-transfected cells were detectable after 7 days.

The results using plasmids delivered with the gold particle system suggested that intracellular RevM10 expression affords partial protection against HIV infection, as evidenced by a longer persistence of RevM10-transfected cells in the peripheral blood. To ascertain whether more durable gene presence could be achieved using an integrating vectoring system, the study was repeated using a retroviral delivery system (36). Similarly, in these experiments, a control vector containing a mutated RevM10 gene was included, which contained a 2 bp-deletion in the translation start codon and a 113-bp inserted sequence at the 3' end of the packaging region to allow discrimination from the RevM10 vector by PCR. Transduction efficiencies ranged from 1% to 10%. As was demonstrated in the prior study, viral challenges *in vitro* demonstrated partial inhibition of HIV replication in cells transduced with RevM10, as evidenced by a 2-3-fold reduction in RT activity. Prior to challenge, cells were selected in G418 for 7 days. At the time of challenge, about 30-50% of the cells were estimated to be gene-modified, which accounts for the partial protective effects noted. Early following cell infusion, both genes were detectable in PBL. Analysis of gene presence over time revealed that cells bearing the RevM10 gene were present at higher frequencies than those bearing the mutant gene. Cells bearing the control vector also became undetectable before those bearing RevM10. RevM10-bearing cells were detectable in 1 patient up to 252 days post-infusion. As compared to gold particle delivery, longer-term gene detection was evidenced using this retroviral transduction method (23 ± 28 vs. 168 ± 74 days; $p = 0.001$). RevM10 message was also preferentially detected over ΔRevM10, as assayed by RT-PCR in PBL stimulated *in vitro* with anti-CD3 and anti-CD28. As expected, no changes in viral load or $CD4^+$ cell counts attributable to the genetic treatment were observed. Cytotoxic responses against Rev before and after treatment were minimal in these patients. These studies suggest that antiviral genetic therapy may confer a

temporary survival advantage to cells bearing protective genes. Large numbers of genetically modified cells would be required to test whether this treatment modality could impact upon the natural history of HIV infection.

7. HUMAN CLINICAL EXPERIENCE WITH ANTI-HIV-1 RIBOZYMES

Ribozymes are RNA molecules with sequence-specific catalytic properties. They can be designed to target essential HIV genes, and can effectively inhibit HIV replication *in vitro*. Similar to antisense, ribozymes may target several steps in the viral life cycle, including "incoming" genomic RNA prior to reverse transcription, as well as viral RNA transcribed from the provirus. Ribozymes can also be designed to target conserved essential regions of the virus, and are capable of repeated interactions with the target RNA. Because they are not translated into proteins, they are considered to be less likely than transdominant proteins to elicit an immunological response. Several clinical studies using ribozymes are ongoing and some preliminary reports are available in abstract form. A T-cell study involving HIV discordant twins employs a hammerhead ribozyme targeted to the HIV-1 *tat* gene (37). This ribozyme, has been shown to inhibit HIV-1 replication (in both laboratory and primary clinical HIV-1 strains) in tissue culture systems (38). Retroviral vectors are used to mediate transfer of the ribozyme and a control neo^R gene into $CD4^+$ T-cells selected from apheresis samples. In 4 patients analyzed to date, transduced cells of each population (ribozyme and control) were found up to 10 months post-infusion. In a study using this ribozyme in CD34+ cells we have reported multilineage gene presence after at 3 months (39). Zaia and colleagues utilized an anti-HIV-1 *tat* and *rev* double hammerhead in a similar $CD34^+$ cell study. Long-term bone marrow cultures of ribozyme transduced cells showed protection from HIV challenge compared to control-transduced cells. In 2 of 4 patients observed for at least 12 months, vector sequences were detectable by DNA PCR in PBMC and/or marrow at 6 months but not at 9 or 12 months post-infusion, and a third patient was positive in PBMC at 4 months post-infusion (40). These studies demonstrate the feasibility of further ribozyme gene therapy clinical research for HIV infection.

8. SUMMARY

Although highly active pharmacological regimens can effectively control viral replication and contribute to partial immune reconstitution, these agents have not been associated to date with eradication of the virus, and their efficacy is undermined by the development of resistant strains that can be transmitted. There is clearly a need for alternative therapeutic modalities. Studies of immune reconstitution in the SCID-hu system and in human drug therapy trials have demonstrated that T-cell development can occur if viral replication is kept in check. Although the current gene therapy experience for AIDS has not resulted in toxicity to date, it has only been associated with preliminary evidence of efficacy, as indicated by an outgrowth of cells carrying protective genes. The available pre-clinical and clinical results support the clinical application of more efficient methods of gene transfer and expression in combination with pharmacological and immune-based therapies.

9. REFERENCES

1. Collier AC, Coombs RW, Schoenfeld DA, Bassett RL, Timpone J, Baruch A, Jones M, Facey K, Whitacre C, McAuliffe VJ, Friedman HM, Merigan TC, Reichman RC, Hooper C, Corey L: Treatment of human immunodeficiency virus infection with saquinavir, zidovudine, and zalcitabine. AIDS Clinical Trials Group. *New Engl J Med* 334, 1011-1017 (1996)

2. Cavert W, Notermans DW, Staskus K, Wietgrefe SW, Zupancic M, Gebhard K, Henry K, Zhang ZQ, Mills R, McDade H, Goudsmit J, Danner SA, Haase AT: Kinetics of response in lymphoid tissues to antiretroviral therapy of HIV-1 infection. *Science* 276(5314), 960-964 (1997)

3. Finzi D, Hermankova M, Pierson T, Carruth LM, Buck C, Chaisson R, Quinn TC, Chadwick K, Margolick J, Brookmeyer R, Gallant J, Markowitz M, Ho DD, Richman DD, Siliciano RF: Identification of a reservoir for HIV-1 in patients on highly active antiretroviral therapy. *Science* 278, 1295-1300 (1997)

4. Lafeuillade A, Chollet L, Hittinger G, Profizi N, Costes O, Poggi C: Residual human immunodeficiency virus type 1 RNA in lymphoid tissue of patients with sustained plasma RNA of <200 copies/mL. *J Infect Dis* 177, 235-238 (1998)

5. Kohn DB, Hershfield MS, Carbonaro D, Shigeoka A, Brooks J, Smogorzewska EM, Barsky LW, Chan R, Burotto F, Annett G, Nolta GA, Crooks G, Kapoor N, Elder M, Wara D, Bowen T, Madsen E, Snyder FF, Bastian J, Muul L, Blaese RM, Weinberg K, Parkman R: T lymphocytes with a normal ADA gene accumulate after transplantation of transduced autologous umbilical cord blood $CD34^+$ cells in ADA-deficient SCID neonates. *Nature Med* 4, 775-780 (1998)

6. Miller DG, Adam MA, Miller AD: Gene transfer by retrovirus vectors occurs only in cells that are actively replicating at the time of infection. *Mol Cell Biol* 10, 4239-4242 (1990)

7. Dunbar CE, Cottler-Fox M, O'Shaughnessy JA, Doren S, Carter C, Berenson R, Brown S, Moen RC, Greenblatt J, Stewart FM: Retrovirally marked CD34-enriched peripheral blood and bone marrow cells contribute to long-term engraftment after autologous transplantation. *Blood* 85, 3048-3057 (1995)

8. Hanenberg H, Xiao XL, Dilloo D, Hashino K, Kato I, Williams DA: Colocalization of retrovirus and target cells on specific fibronectin fragments increases genetic transduction of mammalian cells. *Nature Med* 2, 876-882 (1996)

9. Hanenberg H, Hashino K, Konishi H, Hock RA, Kato I, Williams DA: Optimization of fibronectin-assisted retroviral gene transfer into human CD34+ hematopoietic cells. *Human Gene Ther* 8, 2193-2206 (1997)

10. Pollok KE, Hanenberg H, Noblitt YW, Wendy L. Schroeder WL, Ikunoshin Kato I, David Emanuel D, and David A. Williams DA: High-Efficiency Gene Transfer into Normal and Adenosine Deaminase-Deficient T Lymphocytes Is Mediated by Transduction on Recombinant Fibronectin Fragments**.** *J. Virol* 72, 4882-4892 (1998)

11. Amado RG, Symonds G, Jamieson B, Zhao G, Zack JA: Effects of megakaryocyte growth and development factor on survival and retroviral transduction of T lymphoid progenitor cells. *Human Gene Ther* 9, 173-183 (1998)

12. Barquinero J, Kiem HP, von Kalle C, Darovsky B, Goehle S, Graham T, Seidel K, Storb R, Schuening FG: Myelosuppressive conditioning improves autologous engraftment of genetically marked hematopoietic repopulating cells in dogs. *Blood* 85, 1195-1201 (1995)

13. Abonour R, Einhorn L, Hromas R, Robertson MJ, Srour E, Traycoff CM, Bank A, Kato I, Asada K, Croop J, Smith FO, Williams DA, Cornetta K: Highly efficient mdr-1 gene transfer into humans using mobilized CD34+ cells transduced over recombinant fibronectin CH-296 fragment. *Blood* 92, abstract #2838 (1998)

14. Malech HL, Horwitz ME, Linton GF, Theobald-Whiting N, Brown MR, Farrell CJ, Butz RE, Carter CS, DeCarlo E, Miller JA, Van Epps DE, Read EJ, Fleisher TA: Extended production of oxidase normal neurtrophils in X-linked chronic granulomatous disease (CGD) following gene therapy with $gp91^{phox}$ transduced CD34+ cells. *Blood* 92, abstract #2839 (1998)

15. Korin YD, Zack JA: Progression to the G1B phase of the cell cycle is required for completion of HIV-1 reverse transcription in T cells. *J Virol* 72, 3161-3168 (1998)

16. Naldini L, Blomer U, Gage FH, Trono D, Verma IM: Efficient transfer, integration, and sustained long-term expression of the transgene in adult rat brains injected with a lentiviral vector. *Proc Natl Acad Sci USA* 93, 11382-11388 (1996)

17. Miyoshi H, Takahashi M, Gage FH, Verma IM: Stable and efficient gene transfer into the retina using an HIV-based lentiviral vector. *Proc Natl Acad Sci USA* 94, 10319-10323 (1997)

18. Sutton RE, Wu HT, Rigg R, Bohnlein E, Brown PO: Human immunodeficiency virus type 1 vectors efficiently transduce human hematopoietic stem cells. *J Virol* 72, 5781-5788 (1998)

19. Uchida N, Sutton RE, Friera AM, He D, Reitsma MJ, Chang WC, Veres G, Scollay R, Weissman IL: HIV, but not murine leukemia virus, vectors mediate high efficiency gene transfer into freshly isolated G0/G1 human hematopoietic stem cells. *Proc Natl Acad Sci USA* 95, 11939-11944 (1998)

20.Miyoshi H, Smith KA, Mosier DE, Verma IM, Torbett BE: Transduction of human $CD34^{+}$ cells that mediate long-term engraftment of NOD/SCID mice by HIV vectors. *Science* 283, 682-686 (1999)

21. Corbeau P, Wong-Staal F: Anti-HIV effects of HIV vectors. *Virology* 243, 268-274 (1998)

22.McCune JM, Namikawa R, Kaneshima H, Shultz LD, Lieberman M, Weissman IL: The SCID-hu mouse: Murine model for the analysis of human hematolymphoid differentiation and function. *Science* 241, 1632-1639 (1988)

23.Namikawa R, Weilbaecher KN, Kaneshima H, Yee EJ, McCune JM: Long-term human hematopoiesis in the SCID-hu mouse. *J Exp Med* 172, 1055-1063, 1990.

24.Withers-Ward E, Amado R, Koka P, Jamieson BD, Kaplan AH, Chen ISY, Zack JA: Transient renewal of thymopoiesis in HIV infected human thymic implants following antiviral therapy. *Nature Med* 3, 1102-1109 (1997)

25.Jamieson BD, Zack JA: *In vivo* pathogenesis of a human immunodeficiency virus type 1 reporter virus. *J Virol* 72, 6520-6526 (1998)

26.Koka PS, Fraser JK, Bryson Y, Bristol GC, Aldrovandi GM, Daar ES, Zack JA: Human immunodeficiency virus inhibits multi-lineage hematopoiesis *in vivo*. *J Virol* 72, 5121-5127 (1998)

27.Jenkins M, Hanley MB, Moreno MB, Wieder E, McCune JM: Human immunodeficiency virus-1 infection interrupts thymopoiesis and multilineage hematopoiesis in vivo. *Blood* 91, 2672-2678 (1998)

28.Akkina RK, Rosenblatt JD, Campbell AG, Chen ISY, Zack JA: Modeling human stem cell gene therapy in the SCID-hu mouse. *Blood* 84, 1393-1398 (1994)

29.Burns JC, Friedmann T, Driever W, Burrascano M, Yee JK: Vesicular stomatitis virus G glycoprotein pseudotyped retroviral vectors: concentration to very high titer and efficient gene transfer into mammalian and nonmammalian cells. *Proc Natl Acad Sci USA* 90, 8033-8037 (1993)

30.An DS, Koyanagi Y, Zhao J-Q, Bristol G, Yamamoto N, Zack JA, Chen ISY: High-efficiency gene transduction of human T-cell progenitors. *J Virol* 71, 1397-1404 (1997)

31.Bonyhadi ML, Moss K, Voytovich A, Auten J, Kalfoglou C, Plavec I, Forestell S, Su L, Bohnlein E, Kaneshima H: RevM10-expressing T cells derived in vivo from transduced human hematopoietic stem-progenitor

cells inhibit human immunodeficiency virus replication. *J Virol* 71, 4707-4716 (1997)

32. Donahue RE, Bunnell BA, Zink MC, Metzger ME, Westro RP, Kirby MR, Unangst T, Clements JE, Morgan RA: Reduction in SIV replication in rhesus macaques infused with autologous lymphocytes engineered with antiviral genes. *Nature Med* 4, 181-186 (1998)

33. Malim MH, Tiley LS, McCarn DF, Rusche JR, Hauber J, Cullen BR: HIV-1 structural gene expression requires binding of the Rev *trans*-activator to its RNA target sequence. *Cell* 60, 675-683 (1990)

34. Woffendin C, Yang ZY, Udaykumar, Xu L, Yang NS, Sheehy MJ, Nabel GJ: Nonviral and viral delivery of a human immunodeficiency virus protective gene into primary human T cells. *Proc Natl Acad Sci USA* 91, 11581-11585 (1994)

35. Woffendin C, Ranga U, Yang Z, Xu L, Nabel GJ: Expression of a protective gene-prolongs survival of T cells in human immunodeficiency virus-infected patients. *Proc Natl Acad Sci USA* 93, 2889-2894 (1996)

36. Ranga U, Woffendin C, Verma S, Xu L, June CH, Bishop DK, Nabel GJ: Enhanced T cell engraftment after retroviral delivery of an antiviral gene in HIV-infected individuals. *Proc Natl Acad Sci USA* 95, 1201-1206 (1998)

37. Carr A, Cooper D, Sun LQ, Gerlach W, MacPherson J, Ely J, Symonds G: A phase I gene therapy study showing safety, feasibility and sustained survival of anti-HIV-1 transduced $CD4^+$ T lymphocytes. 6^{th} Conference on Retroviruses and Opportunistic Infections, Chicago, IL, Abstract #361 (1999)

38. Sun L-Q, Pyati J, Smythe J, Wang L, MacPherson J, Gerlach W, Symonds G: Resistance to human immunodeficiency virus type 1 infection conferred by transduction of human peripheral blood lymphocytes with ribozyme, antisense, or polymeric trans-activation response element constructs. *Proc Natl Acad Sci USA* 92, 7272-7276 (1995)

39. Amado R, Rosenblatt J, Zack J, Reier A, Ely J, Symonds G, Mitsuyasu R: A phase I gene therapy trial of autologous CD34+ cells transduced with an anti-HIV ribozyme. 6^{th} Conference on Retroviruses and Opportunistic Infections, Chicago, IL, Abstract #17 (1999)

40. Zaia JA, Rossi JJ, Ito JI, Castanotto D, Li S, Yam P, Bauer G, Valdez P, Rice CR, Rothchild JC, Li H, Banh VN, Sauter S, Peters G, Parker V, Jolly DJ, Sniecinski I, Forman SJ, Kohn DB: One year results after autologous stem cell transplantation using retrovirus-transduced peripheral blood progenitor cells in HIV-infected subjects. *Blood* 92, abstract #2744 (1998)

Key words: AIDS, HIV, Animal, Model, Treatment

Send correspodence to: Dr Rafael G. Amado, Division of Hematology/Oncology, UCLA School of Medicine, 11-964 Factor Building, Los Angeles, CA 90095 Tel: 310/825-8419, Fax: 310/825-6192, E-mail: ramado@ucla.edu

Received 3/10/99 Accepted 4/26/99