Gene Therapy for Infectious Diseases

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INTRODUCTION

Gene therapy holds considerable potential for the treatment of both hereditary genetic disorders and infectious diseases. Human gene therapy is defined as the introduction of new genetic material into the cells of an individual with the intention of producing a therapeutic benefit for the patient (4, 7, 90). Gene therapy is being investigated as an alternative treatment for a wide range of infectious diseases that are not amenable to standard clinical management (4, 7, 35, 51, 83, 94, 96, 99, 137, 138, 151). Gene therapy for infectious diseases requires the introduction of genes designed to specifically block or inhibit the gene expression or function of gene products, such that the replication of the infectious agent is blocked or limited. In addition to this intracellular intervention, gene therapy may be used to intervene in the spread of the infectious agent at the extracellular level. This could be achieved by sustained expression in vivo of a secreted inhibitory protein or by stimulation of a specific immune response.

Approaches to gene therapy for infectious diseases can be divided into three broad categories: (i) gene therapies based on nucleic acid moieties, including antisense DNA and RNA, RNA decoys, and catalytic RNA moieties (ribozymes); (ii) protein approaches such as transdominant negative proteins (TNPs) and single-chain antibodies; and (iii) immunotherapeutic approaches involving genetic vaccines or pathogen-specific lymphocytes. It is further possible that combinations of the aforementioned approaches will be used simultaneously to inhibit multiple stages of the viral life cycle. The extent to which gene therapy will be effective against infectious agents is the direct result of several key factors: (i) selection of the appropriate target cell or tissue for gene therapy; (ii) the efficiency of...
the gene delivery system; (iii) appropriate expression, regulation, and stability of the gene therapy product(s); and (iv) the efficiency of the inhibition of replication by the gene inhibition product. Since the majority of efforts aimed at developing infectious-disease gene therapy strategies are aimed at inhibiting the human immunodeficiency virus (HIV), anti-HIV gene therapy will be discussed extensively. The underlying concepts described for these anti-HIV studies can be extrapolated to other infectious agents.

NUCLEIC ACID-BASED GENETIC THERAPY

Antisense DNA and RNA

Antisense nucleic acids utilize Watson-Crick nucleic acid base pairing to block gene expression in a sequence-specific fashion. Antisense transcripts can be designed to specifically target various regions of the genome of the infectious agent. Although the mechanism of antisense nucleic acid-mediated inhibition of gene expression is not completely understood, it is hypothesized that RNA duplexes (antisense RNA and target RNA) are degraded by RNase or that the duplex sequence blocks translation of the mRNA. Synthetic antisense DNA oligonucleotides and oligonucleotide analogs which inhibit the replication of several infectious agents have also been designed (1, 3, 5, 12, 29, 44, 48, 62, 73, 76, 89, 97, 139). However, their use for the inhibition of gene expression has been extremely limited because uptake of free oligonucleotides from the extracellular environment is extremely inefficient and because cells can rapidly degrade oligonucleotides, so that any inhibitory activity is transient. Stable intracellular expression of antisense sequences is currently the most efficient method by which antisense nucleic acid technology can be used to inhibit gene expression. A general advantage of the use of antisense RNAs (and also ribozymes, RNA decoys, and DNA oligonucleotides) for gene therapy is their lack of immunogenicity. Consequently, cells engineered to produce antisense genes will not be eliminated by the immune system of the recipient.

Ribozymes

Ribozymes are antisense RNA molecules that have catalytic activity. They function by binding to the target moiety through antisense sequence-specific hybridization and inactivating it by cleaving the phosphodiester backbone at a specific site. The two most thoroughly studied ribozymes are the hammerhead and hairpin ribozymes (the names are derived from their theoretical secondary structures). Hammerhead ribozymes cleave RNA at the nucleotide sequence U-H (H = A, C, or U) by hydrolysis of a 3’-5’ phosphodiester bond, while hairpin ribozymes utilize the nucleotide sequence C-U-G as their cleavage site (15, 20, 143). A distinct advantage of ribozymes is that they are not consumed during the target cleavage reaction and so a single ribozyme can inactivate a large number of target molecules. Additionally, ribozymes can be generated from very small transcriptional units; therefore, multiple ribozymes targeting different genomic regions could be used in the same vector. Due to their unique catalytic properties, ribozymes have the potential to be highly efficient inhibitors of gene expression, even at low concentrations. Ribozymes also have greater sequence specificity than antisense RNA because the target must have the correct target sequence to allow binding and the cleavage site must be present in the right position. However, the functionality and the extent of catalytic activity that ribozymes actually have for their RNA targets in vivo are presently unclear. The development of ribozymes that colocalize in the same subcellular compartment as their target may further increase their effectiveness (135).

A significant limitation of the use of ribozymes for gene therapy is that they are composed of RNA and are therefore susceptible to degradative enzymes (RNase). The development of a ribozyme that is resistant to degradative nucleases should increase the effectiveness of ribozyme-based therapy by increasing the intracellular stability of the ribozymes in the infected cell. Increased stability of ribozymes may be accomplished by creating a high level of secondary structure within the RNA through the incorporation of stem-loop structures on either side of a ribozyme.

RNA Decoys

Overexpressed short RNA molecules corresponding to critical cis-acting regulatory elements can be used as decoys for trans-activating proteins, thus preventing binding of these trans activators to their corresponding cis-acting elements in the viral genome (133, 134). One advantage that RNA decoys have over the other nucleic acid-based strategies is that the decoys are less likely to be affected by variability of the infectious agent because any mutations in the trans-activating protein affect not only binding to the decoys but also binding to the endogenous targets. However, there is some question whether RNA decoy strategies will be as benign to the cell physiology as antisense RNAs, since it has been postulated that cellular factors may associate with them (133). It has previously been demonstrated that a cellular factor termed loop-binding protein is an absolute requirement for Tat-mediated transactivation in an HIV-infected cell (85). It is plausible that RNA decoys do not function by sequestering either the Tat or Rev protein but act by sequestering these cellular factors such as loop-binding protein. The potential sequestration of cellular proteins by RNA decoys raises concern about whether overexpression of RNA transcripts may have deleterious effects on cell viability or function. The safety of intracellular immunization based on RNA decoys will have to be established as rigorously as for protein-based antiviral gene therapy strategies.

PROTEIN-BASED APPROACHES TO GENE THERAPY

Transdominant Negative Proteins

TNPs are mutant versions of regulatory or structural proteins that display a dominant negative phenotype that can inhibit the replication of infectious agents. By definition, such mutants not only lack intrinsic wild-type activity but also inhibit the function of their cognate wild-type protein in trans. Inhibition may occur because the mutant competes for an essential substrate or cofactor that is available in limiting amounts; alternatively, for proteins that form multimeric complexes, the mutant may associate with wild-type monomers to form an inactive mixed multimer (50). A potential drawback of the use of transdominant viral proteins is their possible immunogenicity when expressed by the transduced cells. The engineered cells may consequently induce an immune system response that might result in their own destruction. This may diminish the efficacy of antiviral gene therapy with transdominant proteins. The use of nonviral cellular proteins might overcome this drawback.

Anti-Infectious Cellular Proteins

Proteins that are derived from normal cellular genes and exhibit specific gene inhibitory activity have been identified. These activities may act by preventing the binding of the in-
fectious agent to cells, by binding directly to the regulatory or structural proteins, or indirectly by inducing or repressing cellular factors that in turn influence viral gene expression. One of the most successful in vitro uses of endogenous cellular proteins to inhibit an infectious agent is the use of a soluble version of the HIV receptor CD4 (sCD4) (9, 18, 19, 36, 42, 54, 92, 103, 140). However, the use of sCD4 for the gene therapy of HIV infection in a clinical setting has been disappointing. The intravenous infusion of recombinant sCD4 protein into HIV infected patients failed to show efficacy in phase I clinical trials (55). In contrast to the laboratory strains of HIV, clinical isolates have shown a significantly increased resistance to the neutralizing characteristics of sCD4 (32, 33, 103). However, since these are endogenous cellular proteins, they are nonantigenic. Therefore, cells engineered with these cellular inhibitory genes may not be eliminated by the recipient’s immune system. This is an obvious advantage over the use of genes encoding potentially immunogenic, transdominant viral proteins for gene therapy.

**Single-Chain Antibodies (Intrabodies)**

One of the more novel classes of antimicrobial gene therapies involves the development of intracellularly expressed single-chain antibodies. The single-chain variable fragment of an antibody is the smallest structural domain which retains the complete antigen specificity and binding-site capabilities of the parental antibody. Single-chain antibodies (also called intrabodies) are generated by cloning of the heavy and light chain genes from a hybridoma that expresses antibody to a specific protein target. These genes are used for the intracellular expression of the intrabody, which consists of an immunoglobulin heavy-chain leader sequence that targets the intrabody to the endoplasmic reticulum (ER) and rearranged heavy- and light-chain variable regions that are connected by a flexible interchain linker (39, 84, 88). Since the single-chain antibody cannot be secreted, it is efficiently retained within the ER, probably through its interaction with the ER-specific BiP protein. Intrabodies can directly bind to and prevent gene function or may sequester proteins in inappropriate cellular compartments so that the life cycle of the infectious agent is disrupted.

**Suicide Genes**

Instead of protection by blocking viral replication, efficient protection could also be achieved by selectively killing infected cells. This approach requires the conditional induction of the expression of a “suicide” gene that either directly or indirectly causes cell death upon infection with the virus. Suicide genes can be designed to express toxic proteins under the control of the viral promoter. Examples of suicide genes approaches include engineering cells with a diphtheria toxin A-chain (DT-A) gene, a cytosine deaminase gene, or the herpes simplex virus (HSV) thymidine kinase (tk) gene (21, 31, 49, 53, 98, 102). The HSV tk gene can mediate cell death when growing cells are exposed to antitherapeutic nucleoside analogs such as ganciclovir, and this prodrug is metabolized by HSV TK to a toxic analog. Cytosine deaminase mediates cell death through the conversion of 5-fluorocytosine to the potent cytotoxic agent 5-fluorouracil.

**IMMUNOTHERAPY**

**DNA Vaccines**

An additional nucleic acid-based approach for gene therapy is to attempt to elicit an immune response to native proteins of the infectious agent encoded by transfer of plasmid DNA into cells; in other words, DNA-based vaccinations. The preliminary observations leading to the development of genetic vaccination were made by Wolf et al., when they determined that plasmid DNA encoding marker genes could be expressed following intramuscular injection in mice (158). Although the levels of gene transfer were low, it was determined that the internalized plasmid persisted and was expressed for the life span of the animal. The generation of an immune response to marker proteins encoded by plasmids was demonstrated by two groups using plasmid DNA introduced into the skin of mice by a biologic gene delivery system (136, 155). The development of a protective immune response by immunization with a genetic vaccine was initially demonstrated in mice that underwent intramuscular injection of naked plasmid DNA encoding the internal nucleoprotein of the influenza virus (142). The potential efficacy of DNA vaccination into postmitotic muscle cells has since been demonstrated in a variety of murine and animal models infected with bacterial, viral, or parasitic pathogens. The rationale behind these gene vaccines is to generate both a specific, cytotoxic T-cell response and a humoral response.

There are a number of theoretical advantages of the DNA-based vaccination technology over traditional vaccine strategies. These include (i) the ease of production and preparation of plasmid DNA; (ii) the expression of antigens in their native form, which leads to the efficient generation of both cytotoxic and helper T cells; (iii) the potential to reduce the number of doses of vaccine required to generate a protective immune response; and (iv) the fact that the cells need not be the target cells that are normally infected by the infectious agent. The potential disadvantages of DNA vaccination include (i) accidental introduction of the plasmid DNA into cells other than the intended cell types; (ii) generation of anti-DNA antibodies to the plasmid used for the vaccination; and (iii) random integration of the injected DNA into the target cells.

**Agent-Specific Cytotoxic T Cells**

Another approach to the genetic therapy of infectious diseases is the use of the infected individual’s own cells (CD4, CD8, CD34, or antigen-presenting cells) for restoration of the immune system (47, 70, 113–117). Approaches to direct immunotherapy involve the ex vivo expansion of selected T-cell populations, either CD4 or CD8 lymphocytes, followed by re-infusion of the expanded lymphocyte population into the infected individual. The major area of focus for such adoptive cell therapy for infection has been the use of cytotoxic T lymphocytes (CTL [CD8 cells]). Although the importance of major histocompatibility complex class 1-restricted CD8 CTL in controlling infection by agents such as HIV-1 is not understood, it is clear that early in infection the increase in the number of HIV-specific CD8 cells correlates with the resolution of viremia. These data strongly suggest that MHC class 1-restricted CD8 cells play a role in limiting infection during the acute phase.

**TARGET PATHOGENS FOR ANTIMICROBIAL GENE THERAPY**

**Human Immunodeficiency Virus, the Paradigm for Antimicrobial Gene Therapy**

HIV-1 is a member of the lentivirus family. The virus is composed of an RNA genome which is reverse transcribed into DNA by the reverse transcriptase protein. The integrated viral
genome encodes three sets of viral proteins: the structural proteins (Gag, Pol, and Env), the regulatory proteins (Tat, Rev, and Nef), and the maturation proteins (Vif, Vpu, and Vpr). In theory, the life cycle of HIV-1 can be interrupted by blocking or inhibiting the function of one or more of the key viral proteins or their cis-acting regulatory elements.

**Nucleic acid-based anti-HIV gene therapy.** Preliminary studies demonstrated limited antiviral activity with antisense transcripts to the viral genes tat, rev, vpu, and gag and the primer binding site (23, 60, 111, 112, 125, 126). An in-depth analysis of potential HIV-1 antisense gene sequences was performed in which various antisense RNAs targeted to 10 different regions of the HIV-1 genome were compared for their antiviral effects (118). The antisense gene sequences with the greatest antiviral activity were those that targeted a 1-kb region within the gag gene and a sequence specific for a 562-bp genomic fragment encompassing the tat and rev genes. Further analysis of the antiviral effects of the antisense tat-rev gene fragment has demonstrated a strong inhibition of HIV-1 replication in the Jurkat cell line and primary CD4+ peripheral blood lymphocytes (PBL), but breakthrough replication was observed as the multiplicity of infection was increased (144).

A major limitation to the use of stable expression of antisense sequences as a therapy for HIV-1 infection is that long-term high levels of antisense RNA expression are required for effective inhibition of viral replication. The mechanism through which antisense moieties inhibit gene expression requires that one antisense molecule efficiently bind to one target molecule. The stoichiometry of antisense sequences to target sequences must be at a minimum 1:1, but ratios of 5:1 or 10:1 lead to more effective inhibition of viral replication. Thus, for an antisense gene therapy strategy to be effective, the antisense gene expression must be much higher than the levels of HIV-1 expression. Standard gene therapy vectors containing polymerase II (pol II) promoters often do not produce sufficient levels of antisense sequence to inhibit viral replication. To overcome this problem, vectors containing alternative promoter systems have been developed. A retroviral vector containing a pol III promoter in the context of a double-copy expression cassette (the pol III promoter and antisense gene of interest are contained within each of the two retroviral vector long terminal repeats [LTRs]) potentially could be used to achieve high levels of expression (68, 133, 134). Such a retroviral vector expressing an antisense gene to the transactivation response (TAR) sequence was constructed and shown to inhibit HIV-1 in vitro (28). Another retroviral vector in which the HIV-1 LTR is used as a promoter for the expression of therapeutic genes has been developed (74). This vector would allow for the efficient expression of antisense sequences upon infection of a lymphocyte by HIV-1.

The first investigation into ribozymes designed to inhibit HIV-1 was performed by transfecting a hammerhead ribozyme targeted to the viral gag sequence into CD4+ HeLa cells (120, 122). Upon challenge with HIV-1, the cells were demonstrated to express reduced levels of full-length gag RNA molecules and markedly reduced levels of the gag-derived protein p24. Another hammerhead ribozyme developed to target the 5′ leader sequence of HIV-1 was demonstrated to significantly inhibit HIV-1 replication in T-cell lines (154). This ribozyme has the potential to globally inhibit viral gene expression because the leader sequence is contained within all of the HIV-1-derived RNAs. A hairpin ribozyme also targeted to the leader sequence was demonstrated to efficiently inhibit virus replication but also to inactivate incoming viral RNAs prior to integration into the genome, thereby inhibiting the establishment of infection (38, 101, 164). This ribozyme has further been demonstrated to provide long-term protection from HIV-1 infection in T-cell lines and human PBL in vitro (66, 161). The ability to prevent infection by HIV-1 in the long term may allow uninfected cells to become permanently resistant to HIV-1 infection.

Ribozyme transcriptional units are small enough that several ribozymes could be incorporated into a single vector; thus, ribozymes targeted to several regions of the HIV-1 genome can be delivered within the same cell. Multitarget ribozymes in which a single ribozyme cleaves at multiple highly conserved targets within the HIV-1 genome have also been developed (24). A multitarget ribozyme that targeted regions of the env sequence encoding the HIV-1 envelope has been shown to effectively inhibit the replication of several HIV-1 isolates. The development of ribozymes that localize to the same subcellular compartment as their HIV-1 target RNA may increase their effectiveness as anti-HIV-1 agents. As a test of this strategy, a ribozyme that contained the retroviral packaging signal was demonstrated to efficiently inactivate newly synthesized Moloney murine leukemia virus genomic RNA prior to particle assembly, resulting in a marked decrease in the release of particles (135). A potent limitation to the use of ribozymes for HIV-1 gene therapy is that they are inherently limited in effectiveness due to the high rate of mutation associated with HIV-1 replication. Any disruption of the binding or cleavage sites within the target sequence required by the ribozyme for activity could render the ribozyme totally inactive.

The TAR and Rev response element (RRE) are two cis-acting viral regulatory elements that are the binding sites for the transactivating proteins Tat and Rev, respectively. The antiviral activity of the TAR element decoys was examined by retrovirus-mediated gene transfer into T-cell lines in vitro. Overexpression of the TAR decoys inhibited Tat-mediated transcriptional activation and markedly reduced HIV replication for up to 30 days after challenge with laboratory HIV isolates (68, 133, 134). The TAR decoy inhibition of virus replication results from the decreased binding of the Tat protein to the endogenous TAR elements, which in turn inhibits the transcriptional activation necessary for efficient virus replication. Expression of a polymeric TAR decoy containing up to 50 TAR repeats has been demonstrated to effectively inhibit HIV-1 replication in both T-cell lines and primary lymphocytes (72, 74). Furthermore, the multimerized TAR decoy was shown to efficiently inhibit virus replication in lymphocytes from late-stage AIDS patients (75).

The enhanced expression of RRE decoys by retroviral vectors has also been reported to result in long-term inhibition of HIV replication in human T-cell lines (69). RRE decoys probably function by competing for the binding of REV to the normal RRE sequences. It is clear that the overexpression of TAR and RRE decoys has strong antiviral activity, but there is some question about the effect that the presence of the RNA decoys will have on the normal function of the cell. Both TAR and RRE bind cellular factors in addition to viral proteins. The overexpression of the decoys may also lead to the sequestration of proteins required for normal cell function and thus may have negative effects on cell viability or activity. To avoid this potential problem, an RRE decoy of 13 nucleotides that retained the rev-binding domain but could not bind cellular factors was tested for antiviral activity. This minimal RRE decoy was shown to suppress HIV-1 replication in vitro (68).
lute required for wild-type Rev function (mRNA stabilization and nuclear export). The RevM10 protein contains a 2-amino-acid substitution in this leucine-rich domain (L to D at position 78, and E to L at position 79). RevM10 retains the ability to multimerize and bind to the RRE but, as a result of these mutations, can no longer efficiently interact with a cellular cofactor necessary for Rev function (52, 80). Cell lines stably expressing RevM10 are protected from HIV-1 infection in long-term cell culture assays (6). Transduction of RevM10 into T-cell lines or primary PBL delays virus replication without any negative effects on the cells (40, 157). Recently, it has been demonstrated that RevM10 inhibits HIV-1 replication in chronically infected T-cells (107). A Rev TNP that contains a single point mutation at leucine 78 was developed by our group. It inhibited HIV-1 replication in T-cell lines and PBL when challenged with both laboratory and clinical HIV-1 isolates (28, 145). Another type of Rev TNP was generated by deletion of the nucleolar localization signal sequence (63). This Rev TNP was retained in the cytoplasm and prevented the localization of wild-type Rev to the nucleus by forming inactive oligomers. Two phase I clinical trials based on Rev TNPs have been approved by the Recombinant DNA Advisory Committee (RAC) (Table 1).

The HIV-1 regulatory protein Tat was also used to generate TNPs (6, 46, 91). A Tat TNP was mutated in its protein binding domain (71). Upon transduction into T-cell lines, the Tat TNP inhibited HIV-1 replication for up to 30 days. The mechanism through which this Tat TNP may function is by sequestration of a cellular factor involved in Tat-mediated transactivation. Interestingly, in this study, a retroviral vector that was capable of expressing both a Tat TNP and a Rev TNP was developed. The multi-TNP vector was more effective at blocking HIV-1 replication than were retroviral vectors expressing either the Tat TNP or the Rev TNP alone. This study suggests that the simultaneous inhibition of Tat and Rev may be more effective for HIV-1 gene therapy than the use of vectors expressing single Tat and Rev TNPs. Recently, a double TNP Tat-Rev fusion protein (Trev) was designed in an attempt to inhibit two essential HIV-1 activities simultaneously (2). Trev was generated by substituting the basic amino acids at positions 52 to 57 with neutral amino acids and then linked through a histidine bridge to a Rev TNP (containing a deletion from amino acids 80 to 82 within the leucine-rich domain). Upon transfection or transduction of the Trev gene into T cells, the cells were protected from the cytopathic effects of HIV-1. Simultaneous inhibition of two HIV-1 functions may have potential advantages over single-function TNPs.

TNP moieties based on structural proteins have also been investigated for their anti-HIV-1 functions. The HIV-1 structural proteins (Gag and Env) oligomerize into multimeric complexes during viral assembly. Multimerization makes them ideal candidates for the generation of TNPs. Several Gag TNPs have been investigated, and all of them are capable of inhibiting HIV-1 replication (41, 67, 141, 165). All of the Gag TNPs function by disrupting distinct stages of the viral life cycle, such as viral assembly, viral budding, uncoating of the viral genome, or initiation of reverse transcription. Due to inherently low levels of transcription of gag genes in the absence of the HIV-1 Rev protein, the application of Gag TNPs has been limited (141). The low levels of mutant Gag expressed are insufficient to effectively block HIV-1 replication. It has been determined that the HIV-1 gag gene contains an inhibitory sequence that restricts the expression of gag in the absence of the Rev protein (123, 124). Thus, the Rev-independent expression of Gag TNPs is a difficult antiviral strategy. Env TNPs have been generated as well, but in initial testing showed only low levels of antiviral activity (17). A simian immunodeficiency virus (SIV) Vpx TNP has also been demonstrated to have therapeutic potential for HIV (86).

The T helper cell antigen CD4 functions as the receptor for the HIV through the physical interaction of the HIV envelope glycoprotein gp120 and the CD4 protein. Based on these results, investigators have demonstrated that CD4 protein can effectively bind to and inhibit HIV infection in CD4+ cells (9, 18, 19, 36, 42, 54, 92, 103, 140). The effect of this strategy is to compete for binding of HIV to cellular CD4 with high concentrations of sCD4. For this strategy to be efficacious, a high level of continuous expression of sCD4 will be required. Retroviral vectors expressing sCD4 have been shown to protect T-cell lines from HIV infection in vitro (93, 95). A significant limitation to this strategy is the ability to achieve sufficiently high levels of sCD4 to neutralize HIV effectively.

The Rev protein is a powerful trans-acting regulator of HIV viral gene expression, which is essential for viral replication. This protein is required for the efficient export of unspliced and singly spliced viral mRNAs into the cytoplasm of the infected cell. It has recently been demonstrated that the Rev protein interacts specifically with cellular factors to perform its normal function in the infected cell (121). Eukaryotic initiation factor 5A (eIF-5A) is a cellular factor that binds to the Rev activation domain. The interaction between mutants of the eIF-5A and Rev can effectively inhibit HIV-1 replication in vitro (10). Utilization of the interactions between cellular factors and HIV could provide an additional approach for the development of HIV genetic therapies.

Another cellular protein that inhibits HIV in vitro is human alpha-2 interferon (IFN-α2). To achieve prolonged high concentrations of IFN-α2 and to assess the feasibility of IFN-α2 gene therapy, retroviral vectors expressing IFN-α2 were transduced into T-cell lines that were subsequently challenged with HIV-1 (8). IFN-α2 was conditionally expressed under the control of the HIV-1 LTR and therefore was responsive to Tat trans-activation. The replication of HIV-1 in the transduced cell lines was significantly inhibited, probably as a consequence of transcriptional inhibition. However, effects of IFN-α2 on other steps in the HIV-1 life cycle, such as on assembly and maturation, could not be excluded. Since IFNs are pleiotropic molecules, it will be important to exclude possible side effects in using IFN-α2 for gene therapy.

Expression of an intrabody specific for the CD4 binding region of the HIV-1 gp120 (Env) markedly reduced the HIV-1 replication by trapping the gp160 in the ER and preventing its maturation by cleavage into the gp120-gp41 proteins (25). Intrabodies developed to the Rev protein trapped Rev in a cytoplasmic compartment and blocked HIV-1 expression by inhibiting the export of HIV-1 RNAs from the nucleus (39). Recently, intrabodies containing a simian virus 40 nuclear localization signal sequence were developed to Tat (88). The anti-Tat single-chain antibody blocked Tat-mediated transactivation of the HIV-1 LTR and rendered T-cell lines resistant to HIV-1 infection. Recently, antibody fragments developed against the HIV reverse transcriptase enzyme have been demonstrated to effectively block HIV replication in vitro (79). The potential of single-chain antibodies to act as therapeutic agents in vivo has been proposed (Table 1).

**Anti-HIV-directed immunotherapy.** The use of gene vaccines is another strategy that can inhibit HIV. The therapeutic rationale for this approach is that induction of the augmented CTL response to HIV-1 antigens, such as Env and/or Rev, may lead to the elimination of HIV-1-infected cells (22, 57–59, 65, 152). A reduction in the viral burden may further result in positive therapeutic benefits. Cells transduced with a retroviral
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umbilical cord mononuclear cells with retroviral vectors execute T-cell transformation by stimulating cellular proliferation and cytokine production upon interaction with the T-cell receptor. This receptor leads to cytolytic activity, cell division, and cytokine production upon interaction with an HIV-infected cell (Walker protocol, Table 1).

**Human T-Cell Lymphotropic Virus**

Human T-cell lymphotropic virus type 1 (HTLV-1) is a pathogenic human retrovirus that causes adult T-cell leukemia (ATL) and has also been linked to progressive demyelinating neuropathy. Although HTLV-1 is a type C oncogenic retrovirus whereas HIV-1 is a prototypical lentivirus, these viruses use similar strategies to regulate their gene expression. Consequently, gene therapy strategies similar to those used for HIV-1 infection can be used to inhibit HTLV-1 gene expression. Since the Rex protein of HTLV-1 is essential for HTLV-1 infection, CTLs are expanded in the presence of HIV-1 antigens (Gag peptides, Env peptides, etc.). Individual clones of antigen-specific CD8 cells are generated and reinfused into the HIV-1-infected individual (70, 113). The data to support the use of HIV-1-specific individual CTL clones to limit HIV-1 infection is based on observations that CD8 T cells can inhibit the replication of HIV-1 in human lymphocytes in vitro (148). Several clinical trials based on adoptive cell immunotherapy are under way (Table 1). A novel protocol recently begun involves the redirection of the antigenic specificity of CD8-cell populations through the introduction of genes encoding chimeric T-cell receptors that bind HIV-1-infected cells. This chimeric receptor consists of either a CD4 or single-chain antibody recognition domain linked to the invariant zeta chain of the T-cell receptor. This receptor leads to cytolytic activity, cell division, and cytokine production upon interaction with an HIV-infected cell (Walker protocol, Table 1).

**Influenza Virus**

The influenza virus is a human pathogen that leads to significant morbidity and sometimes mortality in infected individuals and frequently causes devastating epidemics throughout the world. The influenza virus is composed of a negative-stranded segmented RNA genome. Each segment of the genome is a template for the synthesis of a different single mRNA. Neutralizing antibodies can be generated against influenza virus, and they form the basis of the current vaccination strategies. However, since these antibodies recognize the highly polymorphic influenza envelope proteins, they are generally strain specific and do not cross-protect against heterologous influenza strains. This polymorphism accounts for the recurrence of influenza epidemics. In contrast to antibodies, CTLs specific for conserved viral antigens can respond to different strains of virus. The generation of such CTLs requires endogenous expression of the antigen. To overcome the limitation of current influenza vaccines, genetic vaccination with plasmids encoding conserved influenza proteins, such as the nucleoprotein, were introduced by direct intramuscular DNA injection into mice (142). This gene vaccine resulted in the generation of nucleoprotein-specific CTLs and protection from a subsequent challenge with a heterologous strain of influenza A virus, as measured by decreased viral titers in lungs, inhibition of mass loss, and increased survival. Another investigation into the development of a genetic vaccine for influenza was performed by direct comparison of clinically licensed vaccines with several DNA vaccines containing current strains of the virus (37). In this study, DNA-based vaccines elicited antibody against the hemagglutinin surface protein and cell-mediated immunity against the internal nucleoprotein and matrix protein. This study suggests that DNA vaccines are more effective against different strains of influenza virus than are the clinically approved vaccines.

**Human Papillomavirus**

Human papillomavirus (HPV) is a nonenveloped, circular double-stranded DNA virus composed of an undefined number of proteins. Infection with HPV is widespread throughout the population, and HPV has been shown to cause a spectrum of epithelial tumors of skin and mucous membranes. Despite the widespread occurrence of HPV infection as a sexually transmitted disease and its association with malignancy, no specific antiviral agents for HPV are available. Many different strains of HPV have been identified. HPV-1 and HPV-6 appear to be principally responsible for ordinary skin warts and for genital warts, respectively, and about 90% of all oral and cervical carcinomas are associated with persistent infections by specific types of HPV's, for instance HPV-16 and HPV-18. Current treatment of HPV infection is nonspecific and consists primarily of surgical or chemical removal of the wart. However, these therapies are not curative since the viral DNA episome still persists in the basal epithelium of latently infected patients. Transfection of the DNA of HPV-16 or HPV-18 into...
primary human keratinocytes gives rise to immortalized keratinocyte cell lines. These immortalizing functions are mediated by the HPV E6 and E7 proteins, which also seem to regulate the mitotic activity of various cervical cancer cell lines. Endogenously expressed antisense RNA directed against these viral E6 and E7 oncoproteins inhibited the cell growth as well as the tumorigenicity of human cervical carcinoma cells in nude mice (146). In addition, inhibition of the growth of HPV-16- and HPV-18-positive human oral and cervical carcinoma cell lines was observed with either antisense oligonucleotides or antisense RNA (130–132, 159). Similarly, a phosphorothioate antisense oligonucleotide targeted to the translation initiation of both HPV-6 and HPV-11 E2 trans-activator mRNAs was shown to inhibit E2-dependent trans-activation (29). In addition to traditional antisense approaches, HPV-specific ribozymes that specifically cleave cottontail rabbit papillomavirus E7 RNA in vitro have been designed, but it is not known whether these ribozymes effectively inhibit HPV-induced cellular transformation (156). Ribozymes that effectively cleave the RNA transcripts from HPV-16 and HPV-18 have also been developed (26, 27, 77). These data underscore the potential of antisense nucleic acids and ribozymes as anti-HPV gene therapy strategies.

**Hepatitis B Virus**

Hepatitis B virus (HBV) contains a partially double-stranded circular DNA genome inside a nucleocapsid surrounded by an envelope. There are an estimated 300 million carriers of HBV, and the course and outcome of their infections are variable. Most HBV infections are self-limiting and are effectively resolved by the host immune system. A small fraction of HBV infections can become persistent or chronic infections. In these cases, the immune system fails to completely resolve the infection and virus particles can persist for years in the circulation. After a prolonged period of chronic infection, HBV DNA is found integrated into the host genome. Chronic hepatitis cases often lead to cirrhosis of the liver and/or hepatocellular carcinoma. At present, vaccination for HBV is the sole protective measure available against HBV infection. Although IFN-α treatment may be beneficial in some cases, no satisfactory medical treatment for chronic HBV infection is currently available. Antisense oligonucleotides inhibited either human HBV in hepatocellular carcinoma cells or duck HBV in primary duck hepatocytes in vitro as well as in duck HBV-infected Peking ducks in vivo (45, 100). The most effective antisense oligodeoxynucleotide was directed against the gene encoding the surface antigen (pre-S) and resulted in a complete inhibition of viral replication and gene expression in vitro and in vivo. However, HBV replication was reactivated once the antisense gene therapy oligonucleotide was stopped.

To increase the specificity of gene transfer to hepatocytes, anti-HBV chemically modified antisense molecules were coupled to the asialglycoprotein (AGP) via a poly-L-lysine polycationic bridge. These antisense molecule-AGP complexes were targetable to hepatocytes via AGP receptors present on those cells and significantly inhibited HBV gene expression and replication in HBV-transfected HepG2 cell lines (160). Due to the limitations of continuous administration of the antisense oligonucleotides, other vector-based approaches permitting continuous and stable expression of the anti-HBV genes should be investigated. Investigations into the development of an effective DNA-based vaccination strategy for HBV are under way. Intramuscular injection of two chimpanzees with plasmid DNA encoding the major and middle HBV envelope proteins induced strong immune responses with group-, subtype-, and pre-S2-specific antibodies (163). Direct comparison of the immune responses in animals receiving traditional antigen-based HBV vaccines or the DNA-based vaccine suggests that the DNA approach has significant potential for prophylactic immunization against HBV (35).

**Hepatitis C Virus**

Hepatitis C virus (HCV) is an enveloped virus composed of a single-stranded RNA of positive polarity. Most HCV infections are asymptomatic; however, acute infections progress to a chronic state in roughly 50% of patients. These chronic infections often lead to liver cirrhosis and in some cases to hepatocellular carcinoma. The therapeutic options for HCV-infected patients are limited. Conventional treatments such as administration of IFN-α are effective against acute or chronic HCV hepatitis in only a small subgroup of patients. A number of studies have investigated the use of antisense oligonucleotides for the inhibition of HCV replication in vitro (3, 48, 90). Due to the sharp rise in the incidence of HCV infection and the limited treatment options, investigations into the development of a genetic vaccine against HCV have begun (138).

**Herpes Simplex Virus**

Members of the human herpesvirus group include HSV-1, HSV-2, varicella-zoster virus, Epstein-Barr virus (EBV), and cytomegalovirus (CMV). The members of this family are composed of a double-stranded DNA genome contained within a nucleocapsid surrounded by a lipid envelope. The clinical manifestations of HSV infection are very diverse and range from relatively benign skin and genital lesions to potentially lethal encephalitis, neonatal herpes, or disseminated progressive disease in immunocompromised hosts. HSV infection may also be a cofactor in cervical carcinoma. Effective chemotherapy with viral DNA synthesis inhibitors such as acyclovir and vidarabine is available to treat most cases. However, drug-resistant HSV mutant strains have arisen in patients and cause a severe and progressive mucocutaneous disease. Gene therapy may serve as an alternative in patients who are refractory to treatment by chemotherapy. Various groups have now reported that HSV-1 gene expression and replication can be inhibited by chemically modified antisense deoxyoligonucleotides targeted at various regions in the HSV genome (61, 106, 127, 128).

The efficacy of antisense HSV oligonucleotides has been demonstrated in vitro as well as in animal models. Some antisense oligonucleotides were actively inhibiting acyclovir-resistant, TK deletion mutant strains of HSV-1 in vitro. In addition, dominant negative inhibitory mutant proteins were derived from various HSV-1 proteins such as the HSV regulatory proteins ICP0, ICP4, ICP8, and ICP27 and were shown to inhibit viral gene expression and replication (43, 125, 129, 153). The ICP4 transdominant proteins were engaged in nonfunctional complexes with their wild-type counterparts, thereby altering the conformation of their trans-activating domain. The development of a genetic vaccine for HSV is currently under investigation (13, 14, 83). These gene therapy strategies may prove beneficial for the treatment of some life-threatening HSV infections.

**Epstein-Barr Virus**

EBV, a major pathogen in humans, causes acute infectious mononucleosis, as well as lymphoproliferative disease in immune-deficient humans, and is associated with Burkitt’s lymphoma, Hodgkin’s disease, and nasopharyngeal carcinoma. It
has also been linked to other diseases such as chronic mononucleosis, chronic fatigue syndrome, and mononucleosis-like syndrome. EBV has the classic structure of a herpesvirus. The primary route of infection by EBV is via the oropharyngeal epithelium. Viral replication in the epithelial cells permits the establishment of a nonreplicative infection in primary B lymphocytes. The infected B cells express five nuclear antigens (EBNA1, EBNA2, EBNA3A, EBNA3B, and EBNA3C); the latent membrane proteins LMP1, LMP2A, and LMP2B; and two small RNAs (EBERs). During the normal course of primary infection, the host immune system controls the infection by immune system elimination of infected cells that express the nuclear antigens. In those rare cases when the immune system cannot control the acute infection, viral replication can be effectively inhibited by available antiviral drugs. The virus then persists in the latent state as an episome in B cells. The episome is the molecular basis for latent infection, and therefore its replication is the primary target for inhibition by antisense oligonucleotides. The antisense-oligonucleotide strategy focuses on blocking the synthesis of a single protein, the EBNA1 protein, which is the sole protein required for replication of EBV episomes and secondarily the synthesis of other EBV latent products. Phosphorothioate antisense oligodeoxynucleotides with or without sequence specificity complementary to the EBV genes have been found to be potent inhibitors of EBV replication in cell culture (162). Other antisense oligomers that target to several sites within the EBNA1 open reading frame had also been tested (104). Although the use of antisense oligonucleotides seems promising for inhibiting latent EBV infection, further work is needed to prove the efficacy and specificity of this effect. Immunotherapeutic approaches to inhibit the reactivation of EBV in immunocompromised patients receiving bone marrow transplants are currently under investigation as an additional approach to gene therapy for EBV (51).

**Cytomegalovirus**

CMV is a ubiquitous herpesvirus that causes mild or subclinical disease in immunocompetent adults but which lead to severe morbidity or mortality in neonates or immunocompromised individuals. Mortality due to CMV pneumonia is as high as 40% in bone marrow transplant recipients. Disseminated CMV infection is common in AIDS patients and is often associated with gastroenteritis and sight-threatening chorioretinitis. The current drugs for the treatment of CMV infection include ganciclovir and foscarner. However, in immunocompromised individuals, such as AIDS patients, disease invariably recurs after cessation of treatment, necessitating prolonged maintenance therapy. Drug toxicity and the emergence of resistant virus strains associated with long-term therapy have limited the effectiveness of these compounds and demonstrated the need for new treatment strategies. Synthetic antisense oligonucleotides represent a novel alternative to the currently available antiviral drugs. Antisense oligonucleotide was targeted to the polymerase gene and the major immediate-early (IE) transcriptional unit of CMV, which encodes several proteins responsible for the regulation of viral gene expression. Phosphorothioate oligonucleotides complementary to the major IE1 and IE2 regions were evaluated for their antiviral activity (5). Oligonucleotides complementary to the IE2 region exhibited the most potent antiviral activity. One of the oligonucleotides was at least 30-fold more potent than the antiherpetic drug ganciclovir. Potent antiviral activity was observed with an antisense oligonucleotide complementary to the intron-exon boundary of the CMV genes UL36 and UL37 (105).

Other oligonucleotides complementary to the IE1 coding region had been shown to inhibit CMV infection as well (16). Stable expression of an antisense gene cassette to the CMV UL83 almost completely blocked CMV DNA synthesis and extracellular virus production in astrocytoma cells (34).

Another problem associated with CMV infection is the reactivation of latent CMV in immunocompromised individuals, such as those undergoing bone marrow transplantation (108–110). Treatment of bone marrow transplant patients with ganciclovir early after transplantation reduces the incidence of CMV reactivation, but CMV disease can occur later. Also, the use of ganciclovir may be associated with other severe negative side effects. In an attempt to develop an alternative strategy of prophylaxis, investigators have undertaken the development of CMV-specific CTLs to prevent CMV disease in immunocompromised individuals (114–116, 151). The results of this clinical trial indicated that the allogeneic administration of CMV-specific CTLs to bone marrow recipients significantly increased the cytotoxic activity against CMV. The level of cytotoxic activity was similar to the level observed in the donors. This initial clinical trial suggests that the development of CMV-specific CTLs may provide an effective means of preventing bone marrow transplant-associated CMV disease.

**Mycobacterium tuberculosis**

Tuberculosis is defined as a chronic infectious disease of the lower respiratory tract caused by *M. tuberculosis*. Tuberculosis is efficiently spread through aerosols released from an individual with an active infection. Humans infected with *M. tuberculosis* are capable of mounting a significant immune response; however, the immune response is the primary cause of most of the tissue destruction associated with some forms of tuberculosis. Vaccination against tuberculosis is performed by using an attenuated live *Mycobacterium bovis* variant known as bacillus Calmette-Guérin (BCG). Unfortunately, the tuberculosis vaccine is not highly effective at protecting individuals, and it renders future immunological testing for diagnosis significantly more difficult. The current mode of treatment for tuberculosis infection is through the use of antibacterial drugs. However, the widespread use of antibiotics as a treatment for tuberculosis has led to the generation of drug-resistant strains of *M. tuberculosis*. It is these drug-resistant strains that are responsible in part for the recent increase in new cases of tuberculosis.

As a novel approach to the prevention of *M. tuberculosis* infection, several laboratories have begun searching for a better vaccine by using the DNA-based vaccine approach (56, 136, 137). Investigations into genetic vaccines for *M. tuberculosis* have focused on the expression of a single mycobacterial antigen, either heat shock protein 65 (Hsp65) or antigen 85, a secreted component. In preliminary studies, expression of either protein in murine muscle cells generated levels of protection equivalent to that obtained in animals vaccinated with the attenuated live BCG. These preliminary studies suggest that novel approaches to vaccine generation may prove beneficial for generation of an improved tuberculosis vaccine.

**EXAMPLES OF CLINICAL TRIALS FOR INFECTIOUS DISEASE**

As of December 1996, 20 clinical gene therapy protocols for infectious disease had been reviewed and approved by the RAC. Most of the approved clinical trials (18 of 20) are targeted toward the development of genetic therapies for HIV-1 infection; however, the insight gained from these clinical trials...
will have an impact on future clinical trials for other infectious diseases. The clinical protocols can be divided into three categories: (i) gene-marking studies, which are not designed to provide any therapeutic benefit to the patients; they are, however, vital for the analysis of the safety of gene delivery systems and cell transplantation techniques; (ii) inhibition of replication, in which clinical trials are designed for the intracellular inhibition of replication of the infectious agent; and (iii) immunotherapeutic gene therapy strategies, which are aimed at stimulating an immune response for the specific immune system elimination of infected cells.

All of the proposed clinical trials use the technologies discussed earlier, such as TNPs, ribozymes, antisense nucleic acids, single-chain antibodies, or specific CTLs (Table 1). Some examples of clinical trials are presented below.

**Marking of Syngeneic T Cells**

A gene marker study on the safety and survival of the adoptive transfer of genetically marked syngeneic lymphocytes in HIV-discordant identical twins has been initiated by Walker and colleagues (150). The objective of this phase I–II pilot project is to evaluate the distribution, survival, tolerance, safety, and efficacy of infusions of activated, gene-marked syngeneic T lymphocytes obtained from HIV-seronegative identical twins on the functional immune status of HIV-infected twin recipients (Fig. 1).

This protocol represents the initial step in a sequence of studies designed to evaluate the potential value of genetically modified CD4⁺ and CD8⁺ lymphocytes in an attempt to prevent or control HIV infection. This study will provide the initial baseline data needed to prospectively evaluate the fate of activated CD4⁺ and CD8⁺ cells after reinfusion in HIV-infected individuals.

**Transdominant Negative Rev**

Based on the encouraging preclinical data obtained with the Rev M10 transdominant mutant, a clinical protocol was proposed by Nabel et al. whereby CD4⁺ T lymphocytes from an HIV-1 infected individual will be engineered with Rev M10 expression vectors (99). In this study, the efficacy of intracellular inhibition of HIV-1 infection by the M10 transdominant mutant Rev protein will be evaluated. The aim of this proposal is to determine whether the expression of M10 can prolong the survival of PBLs in AIDS patients, thus conferring protection against HIV-1 infection. CD4⁺ T lymphocytes will be genetically modified in patients by using either particle-mediated gene transfer or retrovirus-mediated gene transfer. In each case, a control vector identical to the Rev M10 but with a frameshift that inactivates gene expression will be used to transduce a parallel population of CD4⁺ cells. Retroviral transductions and particle-mediated transfections will be performed after stimulation of CD4⁺-enriched cells with IL-2 and either anti-CD3 or anti-CD28 antibodies. Activation of endogenous HIV-1 is inhibited by the addition of reverse transcriptase inhibitors plus an HIV-specific toxin gene (CD4-PE40). The engineered and expanded cells will be returned to the patient, and the survival of the cells in each group will be compared by limiting-dilution PCR. The effect of Rev M10 on HIV-1 status and immunological parameters will also be evaluated. Results from three patients in whom the M10 vectors were delivered into PBLs by particle bombardment technology have been published (99). These results indicate that there is an increase in the length of survival of PBLs receiving the Rev M10 vector over that of PBLs containing a control vector that contains but does not express M10. The limitation of these preliminary results is that gene expression resulting from particle bombardment is only transient, and thus any differences observed could be due to the transient nature of expression of the two vectors.

A similar clinical protocol for AIDS gene therapy, in which retrovirus-mediated gene transfer is used to deliver antisense TAR and RevTD genes to syngeneic lymphocytes in identical twins discordant for HIV-1 infection, is under way (96). This phase I pilot study is based on the preclinical data obtained with the antisense TAR and RevTD retroviral vectors and on
the adoptive transfer of neomycin-marked syngeneic CD4⁺ T cells in HIV-1-discordant identical twins described above (Fig. 2) (150). In this clinical trial, we will evaluate the safety, survival, and potential efficacy of the adoptive transfer of genetically engineered syngeneic lymphocytes obtained from HIV-seronegative identical twins on the functional immune system status of HIV-infected twin recipients (155).

**Gene Vaccines**

Two similar clinical protocols have been approved by the RAC and Food and Drug Administration to test the safety and potential efficacy of genetic vaccination in HIV-1-infected individuals. In these protocols, HIV-1-infected patient fibroblasts are removed for ex vivo transduction with a potentially immunotherapeutic Moloney murine leukemia virus-based retroviral vector encoding the HIV-1 Env and Rev proteins (designated HIV-IT). The ex vivo genetic vaccination phase I clinical protocol involves three successive doses (and a booster set of three successive doses) of HIV-IT-transduced autologous fibroblasts (Fig. 3). These fibroblasts will be obtained from a skin biopsy specimen and will then be transduced with the HIV-IT vector, selected, irradiated, quality control tested, and returned to the donor. The direct in vivo injection protocol is a phase I placebo-controlled clinical trial involving the administration of the HIV-IT vector. Direct vector treatment consists

FIG. 2. Schematic representation of the clinical trial to evaluate the survival of CD4⁺ PBMC transduced by either a neomycin gene (NEO)-containing retroviral vector or a retroviral vector containing an anti-HIV gene plus the neomycin gene in the discordant-identical-twin model. Total PBMC are isolated by an apheresis protocol from the HIV-negative twin. The PBMC population is enriched for CD4⁺ cells by the immunomagnetic depletion of CD8⁺ cells. The CD4⁺ cell population is divided into two fractions. One fraction is transduced by therapeutic retroviral vector, while the other fraction is transduced with the NEO-containing retroviral vector. Following the transduction and expansion of the CD4⁺ PBMC, the cells are mixed at a 1:1 ratio of transduced cells and reinfused into the HIV-positive twin. The survival of transduced cells is monitored by PCR analysis of genomic DNA isolated from CD4 or CD8 PBMC or lymph node (LN) biopsy specimens. Parameters of HIV replication are monitored to assess the potential efficacy associated with the presence of the CD4⁺ PBMC transduced with the anti-HIV retroviral vector.

FIG. 3. Schematic representation of the clinical trial to evaluate the generation of an immunotherapeutic response to anti-HIV genetic vaccines. In this protocol, a vector containing HIV-IT is injected directly into the muscle of the patient as many as three times. Following intramuscular introduction of the therapeutic vector, the patient is monitored for HIV-specific T-cell responses, HIV clinical parameters, and potential toxicity associated with the protocol.
of a series of 3-monthly intramuscular injections (by using a two-tier dosing schedule). Treated individuals will be evaluated for acute toxicity and for normal clinical parameters, CD4 levels, HIV-specific T-cell responses, and viral load before, during, and after treatment. Preliminary clinical data suggest that HIV-infected patients treated with vector-transduced autologous fibroblasts show augmented HIV-1, Env specific CD8+ CTL responses. It is hoped that the retroviral vector-mediated immunization will result in a balanced in vivo immune attack by HIV-specific CTLs and antibody responses that may eliminate HIV-infected cells and clear cell-free virus from an HIV-1-infected individual.

CONCLUSIONS

A wide variety of infectious-disease gene therapy strategies that are highly effective in vitro have been developed. Significant progress toward the development of genetic therapies against a number of infectious diseases has recently been made. It has been shown that primary CD4 T lymphocytes, including primary patient isolates can be protected from infection with HIV-1 by using gene therapy approaches based on transdominant mutant HIV-1 proteins; antisense nucleic acids, and ribozymes. The recent data obtained from vector-mediated immunotherapy studies are also encouraging since long-term persistence of CTL and cross-protection against heterologous polymorphic HIV-1 strains has been demonstrated in animal models. One of the most exciting areas of research is the development of the DNA-based vaccination strategies. Preliminary investigations have yielded encouraging results for the development of vaccines for a number of infectious agents. Based on these preclinical findings, several infectious-disease gene therapy strategies have received RAC and Food and Drug Administration approval for testing in infected individuals. It is hoped that these clinical trials will be able to address the question whether rendering a cell resistant to infection by gene therapy will have any therapeutic benefit for the patient.

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