Gene Therapy (1997) 4, 179–180

Editorial

Combined strategies for gene therapy of AIDS

The possibility of treating AIDS by gene therapy stems from the consideration that HIV infection, like any viral infection, is a true genetic disorder, resulting from the acquisition of new genetic material via an infectious process. In addition, infection by a retrovirus, such as HIV, involves the integration by viral genes into chromosomal DNA to become a stable, inheritable feature of the cell genome. The lysogenic nature of retroviral infection renders HIV eradication virtually impossible by conventional antiviral drugs such as reverse transcriptase and protease inhibitors, despite recent progress in pharmacological treatment. The major limitations of these and other drugs lie in selective toxicity and viral resistance. Both features pertain to the strict stereospecific discrimination that a small effector antiviral molecule has to operate on the tridimensional structure of a viral target protein. The genetic approach has all the properties required to be more powerful as the expression of a therapeutic gene can provide the cell with a new effector function which is macromolecular in nature. It ensures that in some cases, target recognition can take place by virtue of primary or secondary structure complementarity. Gene therapy (GT) of AIDS, to be efficacious, has to achieve a significant antiviral effect at the site of viral replication. It should as well restore/maintain the immune function by preserving the T cell repertoire, whose failure leads to fatal opportunistic infections. In synthesis, GT of AIDS should work by preventing *de novo* infection of susceptible cells and by suppressing ongoing replication in chronically infected cells. To achieve these goals, two major requirements have to be met. Firstly, the identification of cells or tissues that need to be genetically treated and secondly, the availability of suitable vector systems and therapeutic genes. If the concept of preventing HIV infection includes blocking routes of viral entry and spreading to the body, one should be aware of the monocyte–macrophage lineage cells that inhabit mucosal tissues. These cells are the site of initial HIV replication when infection is acquired by sexual contact. A real prophylactic application of GT is presently inconceivable if a prior demonstration of the therapeutic efficacy of this approach is not obtained in infected individuals. Already infected individuals would benefit from a genetic treatment that prevents viral replication in the main target cell, the $CD4^+$ T lymphocytes, whose exhaustion eventually leads to the immunodeficiency syndrome. Adoptive transfer of genetically modified syngeneic lymphocytes in HIV-discordant identical twins has shown that the $CD4^+$ T cell pool in adults is maintained by the division of mature T cells rather than by differentiation of prethymic stem cells. Similarly, the increases in $CD4^+$ T cells that result from antiviral therapy or IL-2 therapy are derived from the expansion of existing $CD4^+$ cells, rather than from the emergence of new $CD4^+$ cells from the thymus.[1] Thus, once an element of the T cell repertoire has been lost through HIV infection, it is unlikely to be replaced by a stem cell compartment. These findings, from an immunological perspective, point to the importance of early intervention and suggest that genetic modification of peripheral $CD4^+$ cells, rather than that of the $CD34^+$ population, is a rational therapeutic strategy for HIV-infected individuals. A stem cell compartment should be considered instead, when pursuing genetic modification of dendritic cells, monocytes/macrophages and microglial cells. All these elements, which share a common committed progenitor, are responsible for spreading the infection to blood, lymphoid organs and the CNS. Transduction of the above cells as individual elements appears an insurmountable task, due to their widespread distribution within the organism and their diverse pattern of recirculation and homing.

Suitable vectors for genetic treatment of mature, antigen-specific, $CD4^+$ cells or cells of the monocyte–macrophage lineage and progenitors thereof, will have to be capable of chromosomal integration in the absence of cell mitosis. These vectors, in addition, should be endowed with a greater gene transfer efficiency than conventional Moloney prototype vectors. Lentiviral vectors based on HIV-1 and HIV-2 have recently been produced and shown to be efficient in transducing resting blood cells and neuronal cells.[2] Although virus titers are presently low, mainly due to lack of suitable packaging cell lines for stable, *in trans* production of HIV structural components, these vectors have a number of potential advantages.

- The selective tropism for HIV susceptible cells, dictated by the gp120 stereospecific recognition of CD4 and coreceptor molecules.
- The susceptibility of HIV particles to be pseudotyped by other viral envelopes, which results in tropism expansion to include $CD4^-$ cells and stem cells in particular.
- The inducibility of the LTR by the same signals that activate wild-type virus.
- The potential for multiple gene expression associated with the mechanisms of transactivation, alternative splicing and nucleus–cytoplasm transport typical of lentiviruses.

- The likely production of homologous interference with wild-type HIV-1.[3]
- The possibility of provirus deletion following homologous recombination or site-directed DNA integration of the vector driven by a recombinase system.[4]

Meanwhile, as lentiviral or AAV vectors are further tuned up, GT of AIDS will have to depend on *ex vivo* transduction of relevant cells with a new generation of more efficient Moloney vectors. Therapeutic strategies so far adopted include transduction of peripheral lymphocytes and bone marrow cells with a number of therapeutic genes able to suppress HIV infection. The suppressive mechanisms involve prevention of HIV integration; inhibition of proviral gene expression to indefinitely prolong latency; and activation of suicide genes in virally infected cells. Suppression of HIV replication can be obtained through intracellular production of therapeutic RNA and protein molecules, some of which are simultaneously expressed by the same vector. These molecules include TAR and RRE decoys, antisense and catalytic RNAs, peptides with transdominant negative effect, intrabodies and prodrug activating enzymes. With respect to therapeutic peptides, therapeutic RNA has the advantage of being free of immunogenic properties. Therapeutic RNA, moreover, can be produced in the context of a selfish (intron) or physiological molecule (tRNA or snRNA); can have enzymatic activity; can act as a decoy; and localize into selected intracellular compartments. Simultaneous expression of anti-HIV genes (decoy and ribozyme) has been shown to synergyze in producing an antiviral response.[5] These results, although confined to *in vitro* experiments, seem to prove that 'polygene therapy' aimed at the simultaneous inhibition of different viral targets is feasible. Like currently proposed combinations of reverse transcriptase and protease inhibitors, combinations of therapeutic genes, targeted at the HIV genome and structural or regulatory functions, could block the afferent (integration) and efferent (transcription, maturation) phases of viral replication. One can conceivably expect that this approach will prove effective in blocking HIV replication in chronically as well as in *de novo* infected cells, with the net result of a dramatic reduction of viral load *in vivo*.

A not yet proposed treatment of HIV-infected individuals, would consist of an adoptive immunotherapy based on the *ex vivo* transfer of autologous CD4 T cell lines and clones which recognize specific antigens of HIV and of opportunistic infectious agents. These antigen-specific cells can be generated from HIV-infected individuals with high efficiency in the early phases of infection. Cells can be expanded *in vitro* to provide an unlimited source for storage and later reinfusion in patients with CD4 counts below levels that threaten opportunistic infections. The possibility of genetically blocking the establishment of the proviral state in these cells is particularly relevant for the success of the autologous adoptive transfer and for the reconstitution of the immunological repertoire lost in the course of HIV infection. This treatment, especially if associated with antivirals, can particularly benefit individuals in the early phase of viral infection.

Among the many isues of GT of AIDS that remain open, that of viral resistance has not been properly addressed, not even by *in vitro* studies. A relevant clinical and experimental finding is the observation that genetically marked CD8 cells reinfused in AIDS patients can undergo immunoclearance, due to the expression of a foreign gene (*TK, hygro*) in association with MHC I molecules.[6] This points to the use of vectors carrying genes for positive selection different from those of prokaryotic or viral origin. A note of confidence has come from Nabel's clinical trial, demonstrating that GT of AIDS can be safely approached in human beings, with some evidence of effectiveness.[7] Further adoption of gene transfer *in vivo* is clearly warranted and would mainly serve for a better appraisal of toxicity and safety issues as well as for a definition of the more active viral vectors and therapeutic genes.

After an enthusiastic acceptance of GT as the likely panacea for disorders such as cancer, genetic and infectious diseases, a certain skepticism is now pervading the scene. This stems from the consideration of how many patients do really benefit from genetic treatment. Moreover, old and new biosafety and ethical issues are the subject of continuous debate by the scientific community. The present risk of a GT backlash can only be counteracted by solid, basic research studies concerning vector design, homologous gene substitution and regulated-tethered expression of therapeutic genes.

Giorgio Palù
Institute of Microbiology
University of Padova Medical School
Via A Gabelli 63, 35121 Padova, Italy

References

1 Walker RE *et al*. Adoptive transfer of genetically modified cells in identical twins discordant for HIV infection. *2nd National Conference on Human Retroviruses* 1995, LB8 (Abstr.).
2 Naldini L *et al*. *In vivo* gene delivery and stable transduction of nondividing cells by a lentiviral vector. *Science* 1996; **272**: 263–267.
3 Volsky DL *et al*. Interference to human immunodeficiency virus type 1 infection in the absence of downmodulation of the principal virus receptor, CD4. *J Virol* 1996; **70**: 3823–3833.
4 Cholika A, Guyot V, Nicolas JF. Transfer of single gene-containing long terminal repeats into the genome of mammalian cells by a retroviral vector carrying the *cre* gene and the *loxP* site. *J Virol* 1996; **70**: 1792–1798.
5 Yamada O *et al*. A chimeric human immunodeficiency virus type 1 (HIV-1) minimal Rev responsive element-ribozyme molecule exhibits dual antiviral function and inhibits cell–cell transmission of HIV-1. *J Virol* 1996; **70**: 1596–1601.
6 Riddell SR *et al*. T-cell mediated rejection of gene-modified HIV-specific cytotoxic T lymphocytes in HIV-infected patients. *Nature Med* 1996; **2**: 216–223.
7 Woffendin C *et al*. Expression of a protective gene prolongs survival of T cells in human immunodeficiency virus-infected patients. *Proc Natl Acad Sci USA* 1996; **93**: 2889–2894.