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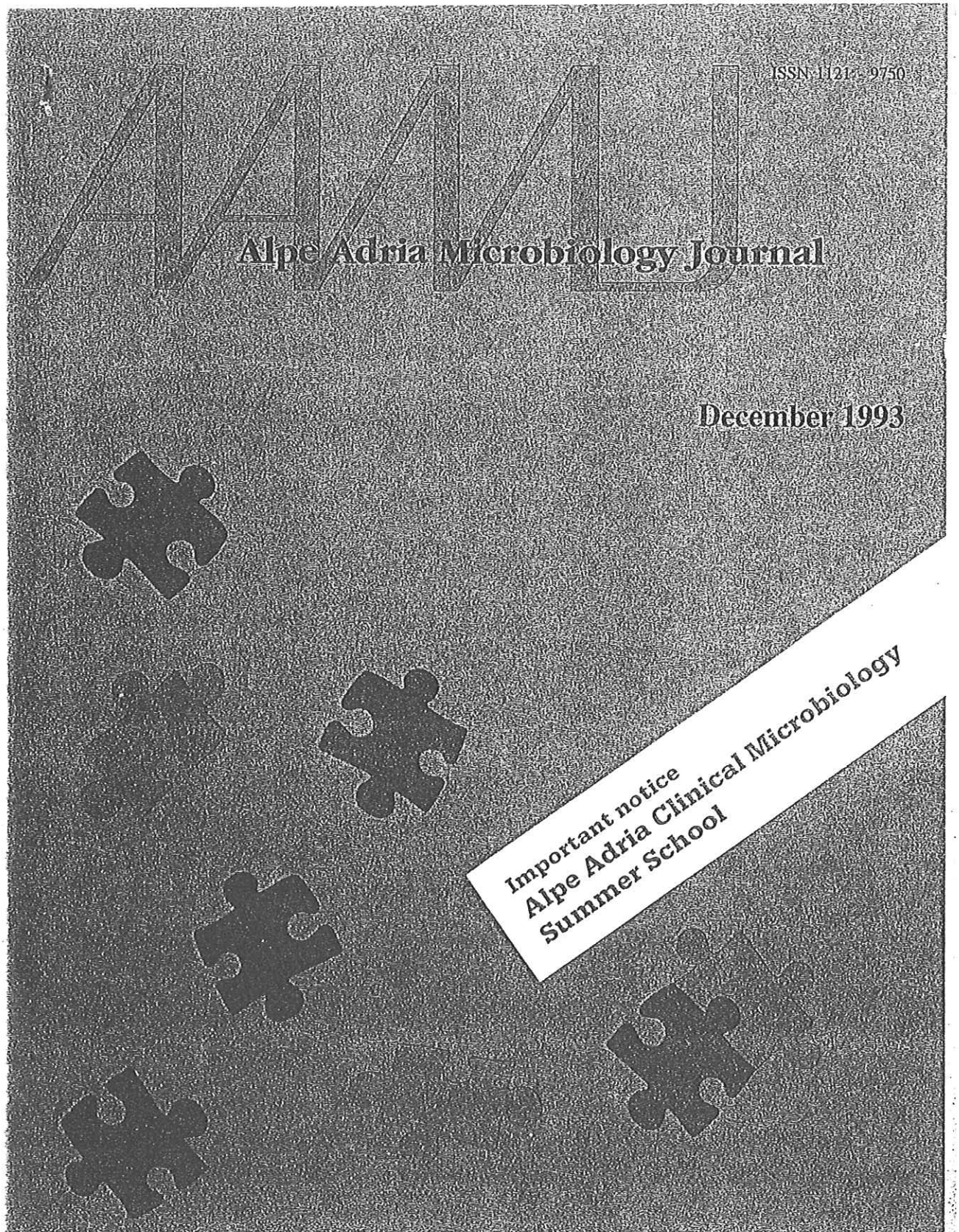

Important notice
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GENE THERAPY AS AN ALTERNATIVE TO CONVENTIONAL THERAPIES FOR AIDS

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The acquired immunodeficiency syndrome (AIDS) caused by the human immunodeficiency virus (HIV) is a chronic disease primarily characterized by the slow depletion of CD4+ lymphocytes. Other major cell types that become infected by HIV are cells of the macrophage/monocyte lineage which also seem to have a role in spreading the infection to other tissues.
Currently, treatment of HIV disease is mainly based on the use of three nucleoside analogs, which interfere with the reverse transcription of the viral genome, namely 3'-azidothymidine, 2', 3'-dideoxyinosine and 2',3'-dideoxycytidine. Although treatment with these agents can delay disease progression and prolong survival, yet it does not represent a cure for AIDS since a complete block of infection is not achieved. One possible explanation for the lack of a curative effect of antiviral analogs could be the inability to maintain adequate drug levels at the site of viral replication over extended periods and the practical impossibility to eradicate the integrated provirus from its latent state. Another explanation for failure of conventional chemotherapy is the emergence of drug resistance. In an attempt to overcome or to prevent selection of drug resistance the use of drug combination has been proposed and clinical trials are currently in progress.
Somatic cell gene therapy is a rapidly developing therapeutic approach for the treatment of a variety of human diseases. The object is to get a new (therapeutic) gene into a cell, either to restore a defective cellular function or to provide the cell with a new function that may help to combat neoplastic or infectious diseases. Different methods of gene transfer are available and the most common consist of physico-chemical and virus-mediated (Table 1) (1). Physico-chemical methods have predominantly been considered for transferring genetic material directly into cells and tissues of the patient. The efficiency of gene expression is high, even if transient, since the DNA sequences transferred remain in a non integrated form.
Viral transfer has been the most widely used, since it permits a more effective

Table 1
Delivery methods for gene transfer into mammalian cells (From Mulligan RC, Science (Vol. 260, 1993), modified)

Method	Application in gene therapy		Expression
	ex vivo	*in vivo*	
Virus-mediated			
Retrovirus	+	?	S
Adenovirus	+/–	+	T
Adeno-associated virus	+	?	S
Herpesvirus	+/–	+	?
Vaccinia virus	+/–	+	T
Poliovirus	+/–	+	T
Physico-chemical			
Ligand-DNA conjugates	–	+	T
Adenovirus-ligand-DNA conjugates	–	+	T
Lipofection	+/–	+	T
Direct injection of DNA	–	+	T
$CaPO_4$ and DEA-dextran precipitation	+/–	–	S
Electroporation	+/–	–	S

T = Transient expression - S = Stable expression

control of the dosage of gene transfer. Retroviruses in particular, have become important tools for efficient gene delivery into eucariotic cells, for they allow the transferred gene to be firmly integrated into the chromosomal DNA of the target cell. Retroviral vectors have been mainly considered for *ex vivo* gene therapy, a procedure by which target cells, removed from the body, are transduced *in vitro* and subsequently reintroduced into the patient.

Promising results from ongoing clinical trials, instituted to assess the application of gene therapy for the treatment of adenosine deaminase deficiency and cancer, have stimulated interest on the feasibility of using gene therapy also for HIV infection. For such an antiviral strategy, it would be necessary to introduce the *therapeutic gene* into cells susceptible to HIV infection. Since HIV enters cells by interaction of its envelope glycoprotein (gp 120) with CD4, a transmembrane protein mostly expressed on helper T-lymphocytes, it has seemed reasonable to consider CD4 expressing T-lymphocytes as potentially important targets for gene transfer.

Among a variety of genetic interventions which have been proposed and are under development (Table 2) (2), there are two basic ones deserving considerations.

The first foresees the expression in CD4+ cells of nucleic acid sequences or gene products that specifically interfere with one or more steps in HIV replication by creating a state of *intracellular immunization*. Candidate therapeutic genes could

Table 2
Targets of gene therapy that have been adopted to control Human Immunodeficiency Virus (HIV) Infection (From Buchschacher GL, JAMA (vol. 269, 1993), modified)

Terapeutic strategy	Molecular target/Proposed mechanism of action
A: Elimination of infected cells	
I. Augmentation of anti-HIV immune response	
gp 160	Stimulation of HIV-specific cellular and humoral immune responses
II. Induced cytocidal effect	
Herpes simplex thymidine kinase	Phosphorylates nucleoside analogs that interfere with DNA synthesis; kills infected cells
Diphtheria toxin	Inhibits protein synthesis; kills infected cells
B: Interference with viral replication	
I. Nucleic acid:	
TAR decoy RNA	Sequester Tat/cell protein complexes; prevent viral gene expression
RRE decoy RNA	Sequester Rev complexes; prevent expression of unspliced/singly spliced mRNA
Anti-sense RNA	Form duplex with viral RNA; interfere with reverse transcription, translation packaging? Result in degradation of RNA hybrids?
HIV-specific ribozymes	Cleave viral RNA
II. Dominant negative mutants	
tat	Form inactive heterodimers? Sequester cellular factors? Prevent viral gene expression
rev	Form inactive protein complexes; prevent expression of unspliced/singly spliced mRNA
gag	Interfere with multimerization during capsid formation?
env	Prevent virus/cell fusion by disrupting hydrophobic fusion pore

encode for antisense and catalytic RNAs, for regulatory and structural viral proteins having a dominant negative effect, or for decoy RNA molecules, which might specifically compete for the binding of essential viral proteins rendering cells resistant to HIV-1 replication. The second genetic intervention is aimed at modifying the host immune response, eliminating HIV-infected cells from an individual.

This can be achieved by transferring into the target cells a gene encoding for a product that induces a cytocidal effect. Such a cytocidal effect could be acting either directly, if the foreign gene product is toxic to infected cells, or indirectly, if the foreign gene product is capable of eliciting a specific immune response which destroys infected cells.

Effort are also underway to increase resistance of helper T lymphocytes to *de*

novo HIV infection through the development and use of HIV-1 vectors (3) that have been engineered as to interfere specifically with the wild type virus. HIV-1 vectors have distinct advantages over conventional murine retroviral vectors, i.e. the specific cell targeting regulated by the gp 120-CD4 interaction, the transcriptional control of the therapeutic gene afforded by the HIV LTR and the possibility to integrate into the chromosomes of non-dividing cells (4). So far, HIV packaging cell lines producing HIV vectors at high titers have not been established. Among the factors that limit the straightforward development of such pakaging cell lines are the complexity the HIV genome and the higly diversified regulation of the HIV replicative cycle. Improvements are needed and new strategies are currently under investigation.

Specific nucleic acid sequences expressed in T cells by murine retroviral vectors have been shown to inhibit HIV replication. An example is the production of TAR molecules using a chimeric tRNA-TAR construct and a double-copy murine retroviral vector. HIV replication was inhibited without affecting cell viability (5) by the overexpressed TAR element that was acting as a decoy for *tat*, a transactivating protein indispensable for virus replication. Preliminary work (6) has demonstrated that transduction of CD4+ cells from HIV seropositive individuals with TAR decoy encoding vectors was conferring HIV-resistance to these cells, although to a less pronounced level than that previously found in a T cell line. Other attempts have involved the expression in T cell of multimerized TAR elements which have lead to a decline in HIV production (7). Similar results were also obtained by overexpression of RRE sequences, which are known to interact with *rev*, a viral regulatory protein (8).

Antisense RNA sequences have also been assayed as potential antiviral agents, which by hybridizing to viral specific transcripts would disrupt one or more steps of HIV replication. Antisense sequences complementary to a region just 5' to the HIV-1 primer binding site (9), or complementary to *tat* (10), and *rev* (10) have often produced a transient inhibitory effect on HIV replication. When antisense RNAs against both 5' and 3' LTRs have been delivered into human hemopoietic and non-hemopoietic cell lines by an adeno-associated virus, the inhibition of viral replication was over 90% (11).

The feasibility of ribozymes as antiviral agents has also been investigated. Ribozymes are small RNA molecules which allow sequences-specific endoribonucleolytic cleavage in a catalytic manner. Different ribozymes have been designed, targetting HIV specific sequences (12). Their activity has been shown to be either comparable to that of antisense molecules, or, in same cases, even less efficient (13).

Another class of genes which have shown to inhibit HIV-1 are those mutant genes responsible for the so called trans-dominant negative effect. Four distinct HIV genes have been used for generation of such a dominant negative effect: the genes for the regulatory proteins, *tat* (14) and *rev* (15), and the genes for the structural proteins, *gag* (16) and *env* (17). The inhibitory effect resulted in on interference with the wild type gene expression, virus assembly and infectivity, respectively.

In order to stimulate anti-HIV immunity, the introduction of the *env* gene, *ex vivo*

into autologous fibroblast cells, which were infused back into the patient, has been attempted as a way to raise an anti-*env* specific immune response. In a mouse model, expression of the envelope glycoprotein by such cells resulted in activation of both HIV envelope-specific cytotoxic T lymphocytes and antibodies (18). Delivery of naked DNA in the form of circular plasmid DNA engineered to express HIV proteins is also being investigated (19). Preliminary results seem promising. This approach offers several advantages including low cost and the ease of preparation of DNA.

Following another therapeutic strategy, aimed at a selective elimination of HIV-infected cells, a HIV-induced toxin gene (the diphteria toxin A chain, DTA) has been expressed in a T cell line resulting in an efficient killing ol infected cells (20).

In a clinical protocol recently approved by the Food and Drug Administration (FDA), cultured, genetically engineered CD8 (T8) lymphocytes, which differentiate into cytotoxic T cells when stimulated by their targets, will be administrered to HIV-positive bone marrow transplant recipients (21). It is hoped that the extra dose of T8 cells, provided by the cultured cells, will help the immune response. Furthermore, the introduction of the herpes simplex virus thymidine kinase (tk) gene into these cells will make the cells sensitive to ganciclovir, allowing a specific elimination of the genetically altered CTL if the need should arise (i.e., massive inflammation).

Although different approaches of gene therapy for HIV infection have been investigated, some of which seem promising for a further development in the form of new antiviral therapy, many problems remain to be solved. So far, gene therapy provides only a short-term cure, since the persistence of the transferred gene is transient. Additionally, it is unclear what role would play the partial inhibition of HIV replication observed in most studies *in vitro* in the context of virus spread and disease progression in an HIV-infected individual. More work is needed to focus on new methods of gene transfer and to improve their efficacy. For a successful application of gene therapy *in vivo*, a more effective use of existing animal models and the development of new models are also necessary.

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CONSIDERATIONS ON THE POSSIBLE ROLE OF VIRUSES IN THE PATHOGENESIS OF TYPE I (INSULIN-DEPENDENT) DIABETES MELLITUS

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In a previous review (1) an up-date of the characterization of the antibody/antigen systems in insulin dependent diabetes mellitus (IDDM) was provided. Here I shall focus on some etiopathogenetic mechanisms likely to contribute to the development of IDDM.

The environment is suggested to play a role in the causation of IDDM. Data supporting this include: the 30-50% concordance for IDDM among identical twins; the seasonal variations in the incidence of new cases of IDDM, with peaks in the autumn and winter months and the coincidence of the peak ages of onset of IDDM with the ages at which children enter primary and secondary schools. This evidence points to common viruses as potential causative factors of IDDM (2). Neonatal events, including breast feeding habits, could also be important.

Despite these indications, there have been few advances in identifying viruses or other agents which may be etiological agents of IDDM. There are reports of increased titres of antibodies to Coxsackie in newly diagnosed IDDM patients, but these results were not confirmed when matched controls were included in the comparative analysis. Similary, no correlation was found between antibodies to the same family of viruses and the presence of islet cell antibodies (ICA) in normal subjects when screening large sections of the population in selected geographical areas. Furthermore, no coxsackie virus envelope proteins are detected in the β-cells of pancreases of newly diagnosed diabetics (3).

CMV sequences have been found in the DNA extracted from lymphocytes of