Review

Gene therapy for HIV

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Introduction

With the advent of highly active antiretroviral therapy (HAART), the therapeutic landscape for HIV infected individuals has changed irrevocably. From what can be likened to a defensive approach to AIDS, juggling prophylactic therapies and treating opportunistic pathogens, HAART has taken us onto the offensive, restoring a sense of optimism to physicians and patients alike in the developed world. It is, however, to quote Churchill only “the end of the beginning.”

It is worth considering why current antiretrovirals are inadequate and what can be done to remedy this situation. Successful antimicrobials target processes that are unique to the pathogenic organism. In fungi, this is often membrane and cell wall synthesis. In bacteria, it is usually cell wall synthesis or the distinctive features of prokaryotic protein synthesis. In viruses, it is predominantly the enzymes. Two of these, reverse transcriptase and protease, are the therapeutic targets in HIV but the actual processes of reverse transcription and protease cleavage are brief events, certainly compared with that of cell wall synthesis in bacteria. The chance of there being subtherapeutic drug levels at critical times during viral replication is, therefore, large. There is additionally no “post-antibiotic effect.” Thus, the imperative for strict adherence to therapy in antiretroviral treatment is as great if not greater than for any other antimicrobial regimen.

The obvious response to this narrow therapeutic window is to seek other targets in the virus life cycle. The difficulty is that most of the remainder of the cycle utilises normal cellular processes and functionally important cellular proteins, interference with which might seriously damage both infected and uninfected cells.

The remaining unique feature of the virus is its nucleotide sequence and, by derivation, the amino acid sequence of its proteins. Reagents which target these specifically would leave cellular functions intact. Thus, the concept of gene therapy against HIV or “intracellular immunisation” as postulated by Baltimore¹ presents itself as a logical solution to this search for alternative treatments. Intracellular immunisation is induction of resistance to infection at the level of the cell rather than at the level of the organism, as achieved by conventional vaccines. The integration of the HIV provirus into the cellular genome defines AIDS as an acquired genetic defect. There is, thus, a certain logic to treating the disease genetically and there is no shortage of therapeutic targets in the viral life cycle.²

Gene therapy is traditionally divided into two types; those where a nucleic acid is the effector molecule and those where it is a protein. A second classification is one based on the mode of action of the genetic therapy. Thus, there are DNA and RNA based antisense molecules, ribozyme and decoy RNAs, transdominant negative proteins, suicide genes, and immunomodulatory proteins. It is the latter classification which will be used in this review.

Nucleic acid based antivirals**ANTISENSE**

DNA oligonucleotides do not fit comfortably into a definition of intracellular immunisation being more akin to conventional pharmaceuticals. Nevertheless, in the knowledge that a nucleotide sequence of only 17 bases is needed for it to be unique within the human genome, the concept of specific binding of a therapeutic to a viral nucleic acid becomes very appealing. Conventional DNA is difficult to handle and relatively labile. Thus, DNA oligonucleotides are usually chemically modified bases containing nuclease resistant linkages such as phosphoramidate or phosphorothioate. The major problem with these agents appears to be tissue and cell penetration. There is probably at least a 10-fold decrease in concentration of an oligonucleotide from the extracellular to the intracellular compartment and a further log order of magnitude loss in concentration from cytoplasm to nucleus where the oligonucleotide has its maximum potential for a therapeutic effect. These problems are discussed by Levin³ and Stein.⁴ This has not prevented commercial companies pursuing clinical trials with GEM®91, a 25 nucleotide phosphorothioate oligodeoxynucleotide directed against the gag initiation codon.⁵

RNA based antivirals

This is the subject of a large recent review⁶ and will be addressed relatively briefly here.

ANTISENSE RNA (SEE FIG 1)

This is RNA complementary to the target RNA which will bind through Watson-Crick bands forming a double stranded structure leading to degradation by cellular enzymes. Unsurprisingly, antisense RNA approaches are concentrated on regions of the HIV genome that are known to have important *cis*-acting (non-coding) functions. Thus, the TAR region/polyadenylation signal,^{7,8} and the packaging signal (Ψ)⁹ have been favourite targets. RNA directed against coding sequences for critical viral proteins such as Tat^{10,11} and Rev, and some of the structural proteins have also been tested.

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If antisense contains special additional ribozyme enzymatic sequence target RNA will be cleaved at a specific site

Figure 1 Antisense and ribozymes.

The majority of these studies have shown a positive effect, inhibiting HIV replication using a variety of techniques for delivery including microinjection or cotransfection of the antiviral with a DNA expressing the viral genes. Cells stably expressing antiviral antisense have also been used to test resistance to an HIV challenge. There are some discrepancies in the results. It would be assumed, for example, that blocking the Tat initiation codon would have a similar effect to targeting the RNA sequence responsive to Tat (TAR). However, this is not the case and this emphasises the difficulty in predicting the effects of these molecules in biological systems as well as deficiencies in the reproducibility of results generated by some of these agents.

RIBOZYME ANTIVIRALS (SEE FIG 1)

Ribozymes bind to their target RNA by sequence complementarity like antisense but they also incorporate a special sequence which acts like a conventional enzyme and cleaves the target RNA specifically, rendering it non-functional. These have predominantly been targeted against similar regions of the genome

Figure 2 Decoy strategy.

to those of antisense as well as the Rev responsive element (RRE),¹²⁻¹⁴ and have a similar reported efficacy. One expectation of a ribozyme is that, being a catalytic agent, it would have a greater effect at a lower concentration as it can recycle and cleave successive target RNAs. This has not always been observed to be the case and it is well recognised that validation of ribozyme activity in vitro does not always correlate with effective function in cells in that some of the effect seen may be due to an antisense effect of the complementary regions of the target and effector RNAs. One principle which does appear to have been validated is the importance of co-localising the effector RNA with its target RNA within the cell.¹⁵ This has been done by comparing targeted with non-targeted ribozymes and showing a significant difference between the effects of the two.

A hairpin ribozyme targeted at the U5 region of the long terminal repeat has been studied in vitro,^{16 17} and in a transgenic mouse model,¹⁸ and is the subject of a Phase I clinical trial.¹⁹ There are also some preliminary results suggesting that an anti-Tat ribozyme containing vector may prolong survival of patient lymphocytes in the face of an HIV challenge.²⁰

DECOYS

The principle of a decoy is to express in a cell an RNA which can adopt a structure identical to an important functional region of the native viral RNA (see fig 2). High level expression of the decoy will sequester away the viral or cellular protein which is supposed to interact with the viral RNA thus preventing normal viral processing. As might be expected,^{21 22} TAR and the RRE have been obvious targets,²³⁻²⁵ and there have been some encouraging in vitro results using this approach. Decoys with a structure identical to the packaging signal (Ψ) have also been tested.²⁶⁻²⁸ However, there is not a clear consistent benefit in terms of inhibition of viral replication in all the studies.

Multifunctional antivirals have also been developed combining different RNA and in some cases protein based antiviral modalities.²⁹⁻³²

Protein based therapies

TRANSDOMINANT PROTEINS

A transdominant protein is one which interferes with a viral process by being a similar but mutated version of the wild type protein. Examples of this include a structural protein which joins in the assembly of a multimeric complex but by its incorporation prevents further binding of additional subunits (see fig 3). Alternatively, a protein which in its native state has a binding and an activation domain may have the latter mutated such that it will still bind but, in doing so, occludes the target from binding the native protein, thus blocking the relevant process. In both these cases, a small number of units are able to overcome the effect of a large number of wild type molecules, hence the designation of transdominant inhibition. The best studied of these in HIV is the Rev M10 dominant mutant,^{23 33} which has been shown to inhibit laboratory and clinical viral

Figure 3 Dominant negative inhibition.

isolates. The M10 mutation is in the nuclear export signal of Rev and, thus, probably interferes with the ability of Rev to transport RNA out of the nucleus. Rev M10 has progressed into Phase II clinical trials³⁴ and experimental results so far suggest a prolonged in vivo survival of cells expressing the Rev M10 as opposed to those expressing an inactive M10 variant. The cellular half life has been extended to a maximum of 15 days which is a relatively modest improvement. Along the same lines, Sam68, a cellular homologue of Rev, has been mutated with the aim of forming a non-functional complex with Rev.³⁵ One danger of mutating cellular proteins is the potential of interfering with their critical cellular functions as well.

Tat transdominant proteins³⁶ and combined Tat/Rev transdominants³⁷ have also been assessed. Other viral proteins, including structural proteins,^{38, 39} and Vpr⁴⁰ and Nef, have also been explored⁴¹ as potential targets. One other difficulty with using proteins as inhibitors is that they themselves may excite an immune response and lead to elimination of cells expressing the "protective" elements. Furthermore, one report has identified potential enhancement of infectivity if inappropriate "transdominants" are used.⁴²

INTRACELLULAR ANTIBODIES

Antibodies have exquisite specificity in binding to their molecular targets and expression of a truncated form of the antibody, which will remain inside the cell, has been used to target viral proteins, preventing viral export. An anti-Tat antibody has been explored⁴³ and work is ongoing using antibodies against the viral envelope and reverse transcriptase enzyme. Similarly, downregulation of cell surface receptors such as CXCR4⁴⁴ to prevent viral entry is a potential approach using this methodology. However, again, there may be a danger in inhibiting expression of a receptor which has critical signalling functions or is essential for cell survival.

SUICIDE GENES

An attractive option is to incorporate into a cell a promoter that will be activated by the viral transactivator, Tat (the viral LTR), driving a gene such as a toxin or the thymidine kinase gene, which in the presence of the drug ganciclovir is lethal to the cell.^{45, 46} Although this is appealing in theory, the specificity of the viral long terminal repeat is relatively poor and it can be activated by a number of other viral and cellular transactivators. Until exquisite specificity of expression is achieved, this is likely to lead to the destruction of antiviral expressing cells in a rather non-specific way.

INTERFERONS

Interferons are natural antiviral substances produced in response to viral infection and other stimuli. A number of studies have explored the possibility of inhibition of viral replication by incorporating the interferon α gene under the control of the HIV-1 promoter⁴⁷ or using the same promoter to drive interferon induced antiviral enzymes such as PKR.^{48, 49} Continuous low dose interferon β expression has also been explored and appears to mediate a general and quite powerful suppression of viral replication.^{50, 51} In a mouse model, it appeared to have no adverse effect on the transplanted cells of the human immune system.

Conclusion

Gene therapy has, to date, been a minority interest in the treatment of HIV infection. In many ways, it is potentially more of a challenge than is treatment of conditions such as single gene defects because the therapy is aimed against something that fights back.

The human race has, like other species, evolved to protect itself against many invading pathogens and, given time, particularly in areas of high disease endemicity and low access to current antivirals, such as sub-Saharan Africa, one may see in the relatively near future such genetic selection beginning to occur. Gene therapy might be considered as a method of mimicking this process and speeding it up hugely by administration of effective, non-toxic but, in this case, non-heritable genetic modulators. Genetic antiviral agents and DNA based vaccines (which it has not been possible to review in this brief article) hopefully will turn out to be simple, non-toxic, low cost, and infrequent therapies available to individuals throughout the world, not just limited to those in the wealthy West.

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