Herpes Simplex Viral Vectors for Therapeutic Gene Delivery to Ocular Tissues

Recent Breakthroughs in the Molecular Genetics of Ocular Diseases

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In recent years, ophthalmology has benefited from a more complete understanding of the molecular genetics of a number of ocular diseases. As examples, genetic linkage studies suggest that a subset of open-angle glaucoma may be genetically determined and the incriminated gene linked to chromosome 1q21-q31.1 More than 50 different mutations in the human rhodopsin gene have been identified in various forms of autosomal dominant retinitis pigmentosa and in one type of autosomal recessive retinitis pigmentosa (Fig. 1). Missense mutations or deletions in the peripherin/RDS gene (coding for a photoreceptor-specific outer segment membrane protein) have been found in patients with autosomal dominant retinitis pigmentosa2 as well as butterfly-shaped pigment dystrophy of the fovea,3 and a null mutation in this peripherin/RDS gene has been associated with retinitis punctata albinescens.4 Mutations in the gene encoding the rod cGMP phosphodiesterase have been recently identified in autosomal recessive retinitis pigmentosa.5 These advances have been speeded by the development of highly polymorphic markers that have expedited localization of disease-causing genes by genetic linkage studies. In addition, the development of pertinent animal models mimicking clinical disease entities (such as the retinal degenerations seen in the rd and rds mouse), along with basic studies on essential elements of normal visual physiology (such as phototransduction) have served to identify candidate genes and gene products that may play a role in inherited ocular disorders of humans.

THE PROSPECT OF HUMAN GENE THERAPY FOR OCULAR DISORDERS. With the mapping and sequencing of disease-associated genetic lesions the identification of genes essential for specific ocular functions comes the potential to move beyond genetic counseling and nonspecific management of many disorders and to consider the possibility of human gene therapy. This can be defined as the transfer of therapeutic genes to ocular tissues by either viral vectors (allowing the virus to serve as a "Trojan horse" to mediate gene transfer), chemical transfer (such as calcium phosphate precipitation ex vivo followed by tissue transplantation), fusion of various ligand-DNA conjugates or DNA-loaded vesicles (for example, liposomes or protoplasts) or physical transfer (for example, microinjection or electroporation). Several of these nonviral techniques rely on receptor-mediated uptake of a DNA-polypeptide ligand, which after entering cells is packaged into endocytic vesicles. The fate of such vesicles is often to fuse with lysosomes leading to nucleic acid degradation unless special means are taken to escape this process.

The eye has certain distinct advantages as a target for virus-mediated gene therapy—including easy accessibility, well-defined anatomy, and translucent media—allowing excellent visual localization of the transfer process. Potential difficulties include problems in localizing and delivering genes to various ocular stem cells (such as stem cells at the corneal limbus, which would have to be the target of gene therapy of the ocular surface for it to be long lasting), and to selected structures in the eye that are at a state of end-stage differentiation (as in the neural retina) and are postmitotic. This lack of, or limited, mitotic ability obviates certain forms of gene therapy that require mitosis for efficient incorporation of the therapeutic gene into the host chromosome, later leading to gene expression.

The approach to human gene therapy will have to be structured to address the particular type of mutation being "rescued." For example, various means of gene transfer in standard orientation may be best suited for specific rescue of conditions caused by either recessive genetic mutations or semidominant null mutations because diseases resulting from dominantly inherited genes will still manifest despite successful gene transfer as long as one copy of the abnormal gene remains. Such dominantly inherited disorders may be more appropriate targets for "anti-sense" gene therapy, attempting to transfer a copy of the abnormal gene in a backward orientation so that its transcript

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Recent Developments

FIGURE 1. Retinitis pigmentosa represents a constellation of diseases that can be inherited in autosomal dominant, autosomal recessive, sex-linked, or simplex fashion. Different genetic mutations within each mode of inheritance result in the common phenotype of photoreceptor degeneration and retinal pigment epithelial migration, characterized clinically by the bone-spicule pattern of fundus pigmentation, shown here.

will result in RNA-RNA hybrid arrest. When bound up in this complex, the aberrant mRNA will not be translated into the mutated gene product. Finally, it may be possible to use gene therapy to treat inherited or genetically determined disorders in which the specific genetic lesion remains obscure. For example, subretinal or intravitreal injection of a number of growth factors, cytokines, and neurotrophins (such as basic fibroblast growth factor, brain-derived growth factor, and interleukin 1β) have been shown to restore specific functions to retinal or retinal pigment epithelial cells and to retard photoreceptor cell death in various animal models of retinal degeneration. The transfer of genes encoding these factors could offer a new generic treatment approach for a variety of vitreoretinal disorders, even where specific genetic defects are not yet defined or the degree of genetic influence uncertain.

Certainly, the prospect of human gene therapy raises numerous issues of safety and ethics. Anderson and Fletcher outlined three minimal requirements as criteria for evaluating human gene therapy protocols: demonstration in animal studies that the new gene can be transferred into the correct target cells and remain there long enough to be effective, demonstration that the new gene will be expressed in the cell at an appropriate level, and demonstration that the new gene will not harm the cell or, by extension, the animal.

SELECTING VIRAL VECTORS FOR GENE THERAPY: THE MEDIATOR IS THE MESSAGE. There are a number of potential advantages to using a viral vector rather than other techniques to mediate gene therapy. The virus may offer some tissue or even cellular tropism to introduce genes selectively into the appropriate sites. In addition, this method affords some control over where the viral and therapeutic DNA may integrate into the human genome or, at least, control over the state of transferred DNA in the cell. In addition, it is possible to capitalize on the fact that most viruses have evolved efficient ways to avoid breakdown of foreign DNA introduced into cells.

A variety of viral vectors has been used in vitro, ex vivo, or in vivo studies, and each may have selective advantages for different forms and targets of gene therapy. Whereas retroviruses allow simultaneous
viruses. A potential disadvantage of AAV as a vector is that the vector may be lost in the genetically engineered vector system. These viruses are readily concentrated and purified and can efficiently infect nondividing and terminally differentiated, postmitotic ocular tissues, such as the retina or corneal endothelium. Furthermore, retroviral vectors are hampered by a propensity to delete partially or completely the foreign DNA insert during subsequent viral replication, as well as by stability problems, frequent rearrangements of their own genetic structure, and promiscuous exchange of sequences with endogenous retroviruses. In addition, there remains a paucity of information available about specific retroviral receptors of the cell surface required for attachment of retroviruses leading to viral cell and tissue tropism.

In contrast to retroviruses, a member of the parvovirus family, the adeno-associated virus (AAV), is able to integrate into the chronosomes of nondividing human cells. Whereas AAV appears to be less efficient and less precise in accomplishing integration into host DNA than retroviruses, it appears stable, highly infectious, and capable of growth to high titer. Frequent integration of wild-type AAV into a segment of human chromosome 19, a region implicated in chromosomal rearrangements in chronic B-cell leukemias, has raised some potential safety concerns, although this propensity may be lost in the genetically engineered vector viruses. A potential disadvantage of AAV as a vector is its limited capacity to incorporate large fragments of foreign DNA.

Adenoviruses are another class of human viruses that have been successfully used as a vector in vivo, targeting airway epithelium and the central nervous system. These viruses are readily concentrated and purified and can efficiently infect nondividing and replicating cells, allowing expression of substantial amounts of gene product. At low to moderate multiplicity of infection, adenoviruses generally do not integrate into the host DNA of infected cells. It, therefore, remains an enigma how persistent infection (and, from the perspective of vector development, persistent exogenous gene expression) with adenovirus is accomplished. There have been concerns that even with so-called replication-defective E1 adenovirus deletion mutants, low levels of adenovirus replication may occur and the requirement for E1 gene expression may not be absolute. Issues of potential toxicity revolve around adenoviral products that shut down host protein synthesis, may be involved in neoplastic transformation, or serve as the target of the host’s immune response. Other viral vectors that have been studied or proposed include herpes simplex virus, vaccinia virus, polio virus, Sindbis, and a variety of other RNA viruses.

THE USE OF HSV-1 AS A VECTOR FOR GENE THERAPY. A strong theoretical basis has already been established for the use of herpes simplex virus type 1 (HSV-1) as a neuronal vector. (For a review, see Breakefield and DeLuca10), and the natural properties of HSV-1 render it most suitable for this purpose. First, HSV-1 can efficiently infect postmitotic neurons, within which it can establish lifelong, transcriptionally active latent infections. Second, HSV-1 is both retrogradely and anterogradely transported within axons. Third, the HSV-1 genome has been completely sequenced and cloned, thus facilitating the generation of recombinant vectors. Finally, the genome of HSV-1 is large (152 kb) and will allow the insertion of up to 8 kb of additional DNA sequences. In spite of these apparently ideal qualities, a number of important issues remain unresolved regarding the possible use of HSV-1 for gene therapy. Some of these issues are discussed below, along with an overview of approaches used for the generation of HSV vectors.

LYTIC AND LATENT INFECTION OF CELLS BY HSV-1. The life cycle of HSV in vivo is marked by two distinct phases. Lytic infection can occur either in neuronal or nonneuronal cells, and all the viral genes are expressed in a strict temporal cascade, with ultimate lysis of the cell and release of progeny virus. By contrast, during latency, which occurs only in neuronal cells, viral DNA persists in the cell nucleus in an episomal form, independent of host chromosomes. Viral gene expression during latency is repressed, with expression restricted to a set of RNA molecules known as the latency-associated transcripts (LATs).11 The LATs are highly abundant and appear to be expressed constitutively in latently infected neurons for the lifetime of the host. The function of these transcripts is not fully understood, but they appear to play some role in the reactivation of the virus from this latent state. Nevertheless, the demonstration of lifelong, transcriptionally active, neuronal latency is probably the most substantive evidence that HSV-1 may well be suitable for neuronal transgenesis.

HSV VECTORS. A key issue in the generation of any vector system either for transgenesis or gene therapy is that the vector must not cause substantive damage to the target cells. All viruses cause some damage to the cells they infect, and HSV is certainly no exception. As noted above, although the HSV-1 can establish latency in neurons, it is also capable of lytic infection in these cells, especially at high multiplicities of infection. Much effort has been put into the genera-
tion of noncytotoxic HSV-1 vectors using two distinct systems, defective viruses and recombinant viruses (Fig. 2).

Defective viruses were originally studied as naturally occurring, defective, interfering particles that arise as a result of serial undiluted passage of viruses.\(^{12}\) The generation of defective virus stocks involves cloning of the gene of interest into a plasmid that contains an HSV-1 origin of DNA replication and an HSV-1 DNA packaging signal. The origin sequences allow the replication of the plasmid (also known as an amplicon) to be driven in eukaryotic cells upon infection with an HSV-derived helper virus. The packaging signal allows this plasmid to be packaged into viral capsids in genomic-length concatemers after replication. Packaging of the plasmid into capsids yields viruses that are termed defective because they do not contain viral DNA and, therefore, they cannot grow in the absence of the helper virus. The helper virus used in this system can be wild-type or, more commonly, a mutant virus with a deletion in any of a number of growth genes that yield a less cytopathic viral stock. Upon serial passage of a mixed helper–defective stock, the ratio of helper to defective virus can approach 1:1. The major advantage of this system is its speed and simplicity, and relatively little virologic knowledge is required. The major disadvantage is the inability to purify defective viruses away from helper viruses. Recombinant viruses have been used extensively to study the viral genes responsible for growth and pathogenesis. For the purpose of vector generation, the gene of interest is cloned into a plasmid containing HSV-1 sequences such that the HSV-1 sequences flank the gene of interest. Transfection of such a plasmid into cells, along with full length HSV-1 genomic DNA, results in homologous recombination and the transfer of the gene of interest into the viral genome, which is then packaged into recombinant viruses. Viral plaques are picked, screened by DNA hybridization, and purified. The major advantage of recombinant viruses is the ability to obtain a purified stock that may help in the control of toxicity. The generation of recombinant virus vectors can be accomplished on a genetic backbone of a virus with preexisting deletions in essential and nonessential growth genes, helping to reduce the cytopathogenicity of the final vector. The major disadvantage is that the generation of recombinants can be technically difficult and time consuming. Neither system, however, can eliminate the problem of cytotoxicity, and the parameters of viral toxicity to cells are still under intense study.

**USE OF HSV-1 VECTORS TO EXPRESS FOREIGN GENES.** A substantial body of literature exists that demonstrates that HSV-1 is capable of delivering reporter genes such as β-galactosidase to neurons and that such genes are expressed to abundant levels (Fig. 3). To date, however, reports of persistent transgene expression from HSV-1 vectors in neurons have been limited to the peripheral sensory ganglia system, which intriguingly is the natural site of latency for HSV-1. Attempts to obtain persistent expression in the central nervous system have not been successful, and this remains a major challenge for the field. It is unclear at this time whether the lack of central nervous system expression is an inherent property of the virus–cell interaction, or whether the use of an appropriate promoter or vector could enable expression to persist. Indeed, it is unclear whether persistent high-level expression of transgenes is appropriate or necessary for gene therapy protocols and whether such expression might be detrimental to the cell. The ideal situation would likely be transgene expression that mimics the expression of the endogenous gene. Some studies in our own laboratories (Leib, Pepose, et al, unpublished observations, 1993) suggest that a rat type II sodium channel promoter is appropriately regulated in the context of a recombinant virus in that the promoter remains neuron-specific and second-messenger inducible (Fig. 3). It may be possible to achieve appropriate regulation of therapeutic transgenes using HSV-1 vectors.

**FUTURE PERSPECTIVES.** Given the demonstrated ability of HSV-1 to introduce and allow expression of foreign genes in postmitotic cells, there is intense interest in their possible use for gene therapy. Much of the work reported to date has focused upon development of vectors for treatment of such brain disorders as Alzheimer’s, Parkinson’s, and Huntington’s diseases. For a number of reasons, however, the retina may be a more feasible target for such pioneering endeavors: The retina is small relative to the brain, allowing delivery of therapeutic vectors to a higher percentage of afflicted cells; the retina is relatively accessible, and the virus can be injected with the target tissue in full view; because our understanding of the genetics of various retinal degenerations is advancing rapidly, many of these disorders seem appropriate for gene therapy approaches; and finally, several well-characterized animal models are available for the study of retinal degeneration and for testing of possible gene therapy protocols. Work ongoing in our own laboratories includes the development of noncytotoxic recombinant HSV-1 vectors that are able to express the rds gene under the control of the opsin promoter.\(^{13}\) This vector, coupled with our subretinal injection procedure that can infect the majority of the photoreceptors of mice with a single injection, will soon be tested in the rds mouse. It is hoped that these
and exciting experiments in other laboratories will pave the way for the development of novel procedures for the treatment of retinal degenerations and set the stage for gene therapy for other ocular disorders.

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