Viral Vectors Take On HIV Infection
Elizabeth D. Mellins, M.D., and Mark A. Kay, M.D., Ph.D.

The lack of robust, classical vaccines against virulent viruses has prompted the search for alternative approaches to the prevention and treatment of infection. A key target in these efforts has been the human immunodeficiency virus (HIV), in which the identification of broadly neutralizing antibodies (bNAbs) in patients led to attempts at passive prophylaxis through the administration of protein. Although this practice appears to be safe in humans, logistical and compliance issues make the maintenance of therapeutic levels of antibodies highly challenging.

The use of gene-transfer therapy to mediate gene expression is a tactic that enables long-term expression of bNAbs or other molecules (including antigens, immune regulatory proteins and signals, and proteins or RNAs) that directly target either the products of the viral genome or the host proteins required for viral entry or replication. The use of a tissue (e.g., muscle or liver) as a depot for the expression of a bNAb against the pathogen essentially bypasses the need to stimulate an endogenous adaptive immune response. For example, a recombinant adeno-associated vector (rAAV) delivered into the skeletal muscle of monkeys expressed a bNAb against envelope glycoprotein 120 (gp120) that protected the recipients against repeated exposure to the simian immunodeficiency virus (SIV). This approach also provided protection against HIV infection and eliminated preexisting HIV infection in a study of mice. Such favorable outcomes of studies in animals have led to a phase 1 trial involving healthy men on the safety and immunogenicity of rAAV1-PG9DP against HIV infection (ClinicalTrials.gov number, NCT01937455), the results of which are expected by early 2016.

However, among the great number of variant gp120 isolates, there are many HIV-1 strains that are resistant or only partially neutralized, even with substantial serum levels of bNAb. This issue is particularly important in the case of the quasi-species that evolve in patients with chronic infections, which suggests that multiple, high-titer antibodies will probably be required for generalized use.

The most conserved regions of the gp120 envelope are the epitopes that bind to the primary receptor (CD4) and a coreceptor, usually CCR5 or CXCR4. The soluble, immunoadhesin form of the CD4 receptor, CD4-Ig, can block HIV infection in culture but has not been successful in the clinic. Another approach, suggested by the amino acid sequences of some bNAbs, involves the use of peptide mimetics in sulfotyrosine-containing coreceptor domains (i.e., the domains of CCR5 or CXCR4), which interact with gp120 in most if not all HIV-1 clades, albeit with a low affinity in the absence of the CD4 receptor. Thus, engineering a protein that binds gp120 to block its binding to both the CD4 receptor and one of its coreceptors is an attractive approach.

Recently, Gardner et al. achieved striking results by using an optimized bifunctional protein that is delivered by means of gene transfer. They used a fusion protein consisting of CD4-Ig and a CCR5 sulfotyrosine-containing mimetic, referred to as eCD4-Ig (Fig. 1), and showed that it had a stronger neutralizing effect on viral activity than that of the best bNAbs (at an order of magnitude of 1 to 2 logs) as measured by viral neutralization against a wide range of HIV-1, SIV, and even HIV-2 clades in cultured cells. The investigators designed two rAAV vectors, one expressing eCD4-Ig and the second expressing an enzyme, tyrosine-protein sulfotransferase, to ensure that eCD4-Ig was properly sulfated. After dual rAAV–vector gene transfer into the muscle, the investigators challenged humanized mice and macaque monkeys with multiple exposures to HIV and SIV, respectively. The rAAV-treated animals were protected against infection,
in contrast with the control animals. Moreover, in
the monkeys, therapeutic levels of eCD4-Ig per-
sisted for at least 40 weeks. These findings
strongly suggest that this particular chimeric
protein may provide long-term protection against
a wide variety of HIV isotypes.

The idea of using gene-transfer vectors to ex-
press serum proteins from skeletal muscle is not
new, but achieving sustained, clinically relevant
levels of the proteins in humans has yet to be
achieved. However, given improvements in rAAV
vectors and sequence alterations in the Fc anti-
body domain that would enhance serum half-life,
and the O-linked sugar of the native CCR5 N-terminal; this CCR5mim1 domain binds gp120 with high affinity and
blocks viral access to cell surface CCR5. The CD4 domains are fused to the human antibody IgG1 Fc domain by a
4-amino-acid linker and the IgG1 hinge, which give the protein conformational flexibility.

In principle, the use of gene therapy to de-
liver passive immunity through antiviral bNAbs
or mimetics can be applied to a wide range of
viral infections. Muscle is easily accessed for the
purpose of localized vector administration, and
specific muscle groups that are amenable to

---

**Figure 1. Structural and Functional Relationships in eCD4-Ig.**

Gardner et al. recently reported using a gene-therapy strategy to counter infection with the human immunodefi-
ciency virus (HIV) in animals. This strategy involves the synthesis, in muscle, of the molecule eCD4-Ig, which con-
stitutes of two domains (d1 and d2) on the CD4 receptor that bind to a highly conserved region on the HIV-1 envelope,
glycoprotein 120 (gp120), and another domain (called CCR5mim1) that mimics the CCR5 coreceptor. The human
antibody IgG1 (fragment-crystallizable) Fc domain improves stability and increases the plasma half-life of the solu-
table CD4 domains. The short CCR5mim1 domain is a 15-amino-acid sulfopeptide that lacks the hydrophobic residues
and the O-linked sugar of the native CCR5 N-terminal; this CCR5mim1 domain binds gp120 with high affinity and
blocks viral access to cell surface CCR5. The CD4 domains are fused to the human antibody IgG1 Fc domain by a
4-amino-acid linker and the IgG1 hinge, which give the protein conformational flexibility.
vector administration can be removed without functional consequence. However, muscle is not a tissue that normally produces circulating proteins, and it contains antigen-presenting dendritic cells that could induce various immune responses (e.g., anti-immunoglobulin responses) that might eliminate the transduced cells or induce autoimmunity. The liver, on the other hand, is an organ that secretes many circulating plasma proteins that might be less immunogenic, but the downside is that delivery to the liver requires systemic administration. In addition, there is no simple means of eliminating expression, should this become necessary. Nonetheless, these are exciting times, and we can expect to see more clinical trials in which similar approaches are evaluated during the next decade. It will be of great interest to see whether a version of the gene-therapy approach or an active classical vaccine will ultimately be the method of choice for the prevention and treatment of these types of viral infections.

Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

From Stanford University School of Medicine, Stanford, CA.


DOI: 10.1056/NEJMci1504232

Copyright © 2015 Massachusetts Medical Society.