Vector-Mediated In Vivo Antibody Expression

BRUCE C. SCHNEPP¹ and PHILIP R. JOHNSON¹
¹The Children’s Hospital of Philadelphia, Abramson Research Center, Philadelphia, PA 19104

ABSTRACT  This article focuses on a novel vaccine strategy known as vector-mediated antibody gene transfer, with a particular focus on human immunodeficiency virus (HIV). This strategy provides a solution to the problem of current vaccines that fail to generate neutralizing antibodies to prevent HIV-1 infection and AIDS. Antibody gene transfer allows for predetermination of antibody affinity and specificity prior to “immunization” and avoids the need for an active humoral immune response against the HIV envelope protein. This approach uses recombinant adeno-associated viral (rAAV) vectors, which have been shown to transduce muscle with high efficiency and direct the long-term expression of a variety of transgenes, to deliver the gene encoding a broadly neutralizing antibody into the muscle. Following rAAV vector gene delivery, the broadly neutralizing antibodies are endogenously synthesized in myofibers and passively distributed to the circulatory system. This is an improvement over classical passive immunization strategies that administer antibody proteins to the host to provide protection from infection. Vector-mediated gene transfer studies in mice and monkeys with anti-HIV and simian immunodeficiency virus (SIV)-neutralizing antibodies demonstrated long-lasting neutralizing activity in serum with complete protection against intravenous challenge with virulent HIV and SIV. These results indicate that existing potent anti-HIV antibodies can be rapidly moved into the clinic. However, this methodology need not be confined to HIV. The general strategy of vector-mediated antibody gene transfer can be applied to other difficult vaccine targets such as hepatitis C virus, malaria, respiratory syncytial virus, and tuberculosis.

INTRODUCTION
The holy grail of human immunodeficiency virus (HIV) vaccine development is an immunogen that elicits antibodies that neutralize field strains of the virus. In recent years, we have gained tremendous insights into the structure and function of the HIV envelope glycoprotein, but limited progress has been made in designing such immunogens. These sobering observations underscore the tremendous hurdles that must be overcome to develop an effective HIV vaccine (1, 2, 3, 4, 5). Foremost among these hurdles is the inability to induce antibodies that neutralize a wide array of HIV field isolates. Such antibodies are rare, and, until recently, only a handful of these antibodies had been isolated (6, 7, 8, 9). Over the past few years, a much larger number of HIV antibodies have been identified that have a much broader range of neutralization and are orders of magnitude more potent than the previously identified group (10, 11, 12, 13). These antibodies were isolated from the high-throughput screening of sera from HIV-1-infected individuals and categorized as “elite neutralizers” based on their neutralization breadth and potency (14). Extensive sequence analysis of these potent, broadly neutralizing antibodies revealed that high levels of somatic mutations were involved to generate the mature antibody (11). Furthermore, the maturation may have involved repeated rounds of antibody selection through HIV antigen interaction, a process that may not be possible to duplicate from a traditional HIV protein subunit or viral vector vaccine.

Given the obstacles required to generate this class of broadly neutralizing antibodies by current vaccine
strategies, one option is to deliver these antibodies by passive immunization. Passive immunization schemes using neutralizing antibodies have protected monkeys from simian-human immunodeficiency virus (SHIV) challenge infections (15, 16, 17, 18, 19, 20). Unfortunately, an injection of antibodies every few weeks is neither practical nor cost-effective as a large-scale human vaccine approach. The vector-mediated gene transfer vaccine strategy eliminates the problems with passive antibody transfer and uses a viral vector to deliver the potent, broadly neutralizing antibodies directly to muscle by gene transfer. In this scheme, the antibody gene of choice is packaged into an adeno- associated virus (AAV) vector, which is then delivered by direct intramuscular injection. Thereafter, antibody molecules are endogenously synthesized in myofibers and passively distributed to the circulatory system (21).

In a proof-of-concept experiment in a large animal model, rhesus monkeys were injected with AAV vectors expressing monkey antibodies able to neutralize simian immunodeficiency virus (SIV) (22). The neutralizing antibodies were detected in the serum for over 6 years following a single intramuscular injection. Furthermore, monkeys were protected from infection and the development of AIDS following challenge with virulent SIV.

The concept of immunoprophylaxis by gene transfer is significant in that it can rapidly move existing and newly discovered molecules that block HIV infection into the clinic. In fact, the development of molecules that inhibit all steps in HIV entry could create a multilayered blockade against HIV infection and provide a shortcut to an HIV vaccine. These molecules could be directed at any or all points of HIV entry including (i) gp120 binding to CD4, (ii) gp120 binding to CCR5, and (iii) membrane fusion. This concept need not be limited to HIV. In fact, the general strategy of "immunoprophylaxis by antibody gene transfer" can be applied to other difficult vaccine targets like hepatitis C virus, malaria, respiratory syncytial virus, and tuberculosis.

**IMPORTANCE OF HIV-NEUTRALIZING ANTIBODIES**

The need for a vaccine is still great, with approximately 35 million people currently infected with HIV, and more than 2 million new adult infections every year. Two earlier vaccine approaches, each targeting a different arm of the immune response, were evaluated in large efficacy trials. Both approaches failed to protect vaccine recipients from infection, and neither diminished viral replication after infection (23, 24, 25, 26). The more recent RV144 trial in Thailand using a canarypox virus expressing HIV proteins in conjunction with a gp120 subunit showed moderate efficacy (31%), indicating that protection may be achievable with the right immunogen (27). Detailed analyses of the RV144 study results revealed two significant correlations with infection among vaccine recipients. The presence of IgG antibodies against V1V2 Env may have contributed to protection against HIV-1 infection, whereas high levels of Env-specific IgA antibodies correlated inversely with infection (28). Very recently, the HVTN 505 trial was stopped for futility. The HVTN 505 trial showed no difference in HIV-1 infections between those recipients who received the vaccine and those receiving placebo (29). Vaccine recipients did generate IgG antibodies to Env; however, the majority were nonneutralizing with low reactivity to the V1V2 antigen (29).

The observations from these clinical trials, that neutralizing antibodies may be essential for an effective vaccine, is reinforced by the large number of new, highly potent broadly neutralizing antibodies (bNAb) that have been identified by using improved screening and sequencing techniques. These new antibodies were isolated by high-throughput screening of sera from healthy HIV-1-infected individuals categorized as “elite neutralizers” based on their neutralization breadth and potency (10, 11, 12, 13, 14, 30, 31, 32, 33). Detailed analyses of these antibodies indicated they are approximately 10- to 100-fold more potent and have an increased breadth compared with the original 4 isolates (6, 7, 8, 9). Furthermore, this new class of antibodies can neutralize HIV-1 through binding to a variety of envelope domains including the CD4 binding site (VRC01, NIH45-46, and PGV04) (13, 31, 34), glycan-containing regions in the variable loops (PG9, PG16, PGT121, and PGT128) (11, 12), and the membrane-proximal external region (MPER) on gp41 (10E8) (30).

Epitope mapping of these new, potent antibodies has invigorated the vaccine field by providing precise regions to target when designing new protein or subunit vaccine antigens to induce bNAb (35). However, even with this new wealth of information at hand, generating bNAb with improved, redesigned antigens may still prove to be problematic. Extensive sequence analysis of these potent broadly neutralizing antibodies reveal that high levels of somatic mutations (as much as 30%) can occur in the generation of the mature antibody (11, 13, 31, 33, 36). Furthermore, the maturation may have involved repeated rounds of antibody selection through HIV antigen interaction. In light of this, several groups have developed novel immunogens, such as glycopeptides or
computation-derived multimerized nanoparticles, that are designed to induce bNAbs (37, 38). These immunogens can bind to both mature bNAbs as well as the receptors of their germline (naïve) B cells, which can trigger the activation and maturation process required to produce a bNAb.

HIV-NEUTRALIZING ANTIBODIES AS A VACCINE

While induction of bNAbs by various next-generation immunization strategies holds promise, the question remains as to the best use of the human monoclonal antibodies that have already been isolated and characterized. One obvious option is passive immunization. Passive immunization using neutralizing monoclonal antibodies has protected monkeys from SHIV challenge infections (15, 16, 17, 18, 19, 20, 39). In a recent study by Moldt et al. (39), they showed that passively administered PGT121 can mediate sterilizing immunity against SHIV in monkeys at serum concentrations that were significantly lower than those observed in previous studies (as low as 1.8 μg/ml). While this study demonstrated the potential for passive immunization with the new class of bNAbs, unfortunately, an injection of antibodies every few weeks is not practical or cost-effective as a large-scale human prophylactic vaccine approach. Given these difficulties, we developed a second option: isolate the representative antibody gene and use gene transfer technology to endow a target host with the gene. In this way, the antibody gene directs endogenous expression of the antibody molecule, and the host (in theory) will now have the antibody in its circulation. Thus, after a single injection, the muscle now serves as a depot to synthesize the bNAbs that are passively distributed to the circulatory system (Fig. 1). The host is now armed with a potent bNAb against HIV-1 that effectively bypasses the adaptive immune system. This is in contrast to the traditional idea of passive immunization whereby the purified antibodies are injected intravenously into the host to provide protection from infection. However, because of the antibody half-life, the levels decline, requiring repeated injections. The obvious advantage is that antibody gene transfer engenders the host with long-term antibody persistence from a single injection due to endogenous antibody expression.

VECTOR EXPRESSION SYSTEMS FOR ANTIBODY GENE TRANSFER

Our chosen vector to deliver the antibody gene is the recombinant adeno-associated virus (rAAV) vector, which is derived from wild-type AAV. AAV is a Dependovirus with a 4.7-kb single-strand DNA genome that contains only two genes (rep and cap) flanked by inverted terminal repeats (ITRs). AAV natural infection is common and has not been associated with any disease. Multiple AAV serotypes have been identified with different transduction efficiencies in different tissues, offering flexibility for gene transfer targets such as muscle or liver (40). rAAV vectors have an established record of high-efficiency gene transfer in a variety of model systems (41, 42). Following injection, the rAAV vector genome can form stable nonintegrating circular episomes that can persist in nondividing cells (43, 44, 45). Because of these features, rAAV vectors have become popular gene delivery vehicles for use in clinical studies for the treatment of diseases such as alpha1-antitrypsin deficiency, cystic fibrosis, hemophilia B, Leber’s congenital amaurosis, lipoprotein lipase (LPL) deficiency, Parkinson’s disease, and muscular dystrophy (46).

rAAV gene transfer vectors are devoid of the endogenous rep and cap genes, and consist of the antibody gene expression cassette flanked by the AAV ITRs (Fig. 2). The ITRs (145 bp each), which are necessary for rAAV vector genome replication and packaging, are the only part of the AAV genome present in the

![Diagram of rAAV vectors for antibody gene transfer.](https://-asmscience.org/MicrobiolSpectrum)
rAAV vectors. One method for antibody expression utilizes a two-promoter system whereby the heavy- and light-chain genes are transcribed independently by using two different promoters and polyadenylation signals within the same rAAV vector genome (Fig. 2) (47). Another method uses a single promoter for expression of both the heavy and light chains, which are separated by the foot-and-mouth-disease virus (FMDV) 2A peptide, which undergoes self-cleavage to produce separate heavy- and light-chain proteins (Fig. 2) (48). The advantage of this system is that the heavy and light chains can potentially be expressed in a 1:1 ratio using a single promoter, which may translate to more efficient expression. However, a potential disadvantage is that the FMDV-2A peptide is derived from a viral sequence and may be immunogenic in the host causing immune clearance of cells expressing the antibody.

Another option is to create an immunoadhesin version of the neutralizing antibody. Immunoadhesins are chimeric, antibody-like molecules that combine the functional domain of a binding protein like a scFv or CD4 extracellular domains 1 and 2 (D1D2) with an immunoglobulin constant domain (Fig. 2) (49), and have been shown to be effective in disease models including HIV, SIV, and influenza (22, 50, 51). A typical immunoadhesin lacks the constant light-chain domain and the constant heavy-chain domain 1 (CH1); however, it can be expressed as a single polypeptide from a single promoter, and forms dimers through disulfide bonding in the hinge region. While immunoadhesins have many attractive features such as efficient expression/secretoin in vivo, they also have some drawbacks. Immunoadhesins may not exhibit the same neutralization breadth and potency as the native antibody. While we have seen cases where a specific immunoadhesin functions identically to its native antibody counterpart, we have also seen an immunoadhesin become 10-fold less potent at neutralizing HIV-1 (unpublished observation). Thus, immunoadhesins must be fully characterized and compared with the native antibody from which they were derived before consideration as a vaccine. Another drawback to using immunoadhesins is possible immunogenicity. Immunoadhesins are not naturally occurring proteins and may contain amino acid linkers connecting the variable domains (Fig. 2), which could trigger an immune response leading to the loss of expression. However, it should be noted that Enbrel (etanercept), an immunoadhesin consisting of the tumor necrosis factor receptor fused to IgG1-Fc, was well tolerated in patients for long-term treatment (10 years) of rheumatoid arthritis (52).

**PROOF-OF-CONCEPT STUDIES IN ANIMAL MODELS**

We first developed the concept of using antibody gene transfer in 2002 (47). Because of the significant obstacles that confronted both active and passive immunization strategies, we began to explore an alternate strategy to generate serum antibodies that neutralize primary isolates of HIV-1. At that time, only a few monoclonal antibodies existed (6, 7, 8, 9). We chose to express IgG1b12 to test the feasibility of antibody gene transfer using rAAV vectors. To generate the IgG1b12 expression construct, the IgG1b12 heavy and light chains were expressed independently in the same rAAV vector using the dual-promoter system. The resulting vector was injected into the quadriceps muscles of immunodeficient mice (to avoid immune responses to human IgG). IgG1b12 was expressed in mouse muscle (confirmed by histochemical staining), and biologically active antibody was found in sera for over 6 months (47). Characteristic biologic activity was determined by HIV neutralization assays against IgG1b12 sensitive/resistant viruses. This study provided the first evidence that: (i) rAAV vectors transferred antibody genes to muscle; (ii) myofiber produced antibodies; (iii) antibodies were distributed to the circulation; and (iv) such antibodies were biologically active.

While these initial findings supported the hypothesis that antibody gene transfer to the muscle can produce systemic levels of HIV-neutralizing activity without the need for an active humoral response, it was difficult to determine if this activity would translate to protection from a challenge infection. Therefore, our next goal was to test our hypothesis in a macaque challenge model, but we were faced with the problem that the human antibody IgGb12 was viewed as foreign in a macaque, triggering an immune response that lead to the elimination of IgG1b12 expression. We then turned to using rhesus-derived antibodies by taking advantage of native macaque SIV gp120-specific Fab molecular clones that had been derived directly from SIV-infected macaques (53). When designing the antibody gene transfer vectors, we chose to express the Fabs as immunoadhesins, which in pilot experiments in mice were superior to single-chain (scFv) or whole-antibody (IgG) molecules with respect to steady-state serum concentrations (unpublished data).

For the macaque experiments, we constructed immunoadhesins derived from two different SIV Fab fragments (4L6 and 5L7), as well as a third immunoadhesin containing the rhesus CD4 D1D2, which was modeled
after CD4-Ig fusion proteins (54). All of the constructs neutralized in vitro the proposed SIV challenge stock (SIVmac316), indicating that the immunoadhesins were functioning like the original Fab clones (22). The 3 immunoadhesins were injected into 3 monkeys each (for 9 total), followed by an intravenous SIV challenge 4 weeks later, including 6 naïve controls. Immunoadhesin expression levels were as high as 190 μg/ml at the time of challenge (4 weeks postinjection) and peaked around 6 months with levels reaching 400 μg/ml in some animals (22). Overall, 6 of the 9 monkeys receiving the immunoadhesins were completely protected after challenge, while all 6 naïve controls became infected. Analysis of the 3 monkeys from the immunoadhesin group that became infected revealed that these specific animals had developed an immune response to the immunoadhesin by 3 weeks postinjection, suggesting a correlation between an immune response to the immunoadhesin and failure to protect from infection. We have performed longitudinal studies of the protected monkeys, which are now over 6 years postinjection. Immunoadhesin levels dropped to a stable level of approximately 20 μg/ml, which has persisted for over the past 4 years. The monkeys have remained negative for SIV infection and have not developed an immune response to the immunoadhesins (unpublished observation). Thus, this crucial study was instrumental in proving the concept of vector-mediated gene transfer as a viable HIV vaccine.

More recently, other investigators performed rAAV vector-mediated gene transfer expression/challenge studies, which they called vectored immunophylaxis (VIP) (55). They expressed the native, full antibodies of 2G12, IgG1bI2, 2F5, 4E10, and VRC01 using the single-promoter FMDV-2A system. Following intramuscular rAAV injection in mice, antibody expression levels greater than 100 μg/ml were observed for at least 12 months. Using a humanized mouse model, they further showed that these rAAV vectors provided protection following HIV challenge, with antibody serum levels as low as 8.3 μg/ml (antibody VRC01). These encouraging results reinforce the efficacy of the antibody gene transfer approach, especially when potent antibodies such as VRC01 are used. Taken together, these murine and primate studies show that vector-mediated antibody gene transfer can bypass the adaptive immune response and engender the host with antibodies that provide protection from infection. Furthermore, antibody expression can persist several years following a single injection, suggesting that long-term protection is possible.

ANTIBODY GENE TRANSFER FOR HIV-POSITIVE INDIVIDUALS

While antibody gene transfer shows great promise for providing protection from HIV infection, one obvious question is whether this strategy can also be used for antibody therapy in HIV-positive individuals. To answer the question, HIV-infected humanized mice received an intravenous injection of a rAAV (serotype 8) vector expressing bNAb 10-1074 (56), which targets the base of the V3 stem of gp120 (57). These mice maintained a high level of antibody 10-1074 expression of around 200 μg/ml for the entire length of the 67-day observation period. During this time, 6 of the 7 mice in the group were able to control HIV plasma viral loads, whereas 1 mouse exhibited viral escape. As seen with escape mutants from passive immunization studies (56, 58), sequence analysis of the gp120 of these escape viruses revealed mutations in the 10-1074 binding site that conferred resistance to the antibody. It remains to be seen if simultaneously administering rAAV vectors expressing multiple bNAbs could dramatically reduce or even possibly eliminate the generation of escape mutants. Furthermore, long-term studies will be required to see if escape mutants could arise over time, even in the presence of multiple antibodies. These results in humanized mice suggest that using vector-mediated gene transfer to deliver bNAbs to HIV-infected individuals could be a viable option, possibly even used in conjunction with standard antiretroviral therapy (ART). An overriding theme is that multiple bNAbs would be required to provide the selective pressure to avoid viral escape mutants. Multiple antibodies could target different gp120 domains such as the exterior loops, CD4 binding site, and MPER. Furthermore, multiple antibodies could be used that target different stages of viral entry including CD4 binding, CCR5 binding, and membrane fusion. Of course, this strategy of the simultaneous use of multiple antibodies against multiple viral targets or stages of entry could also be applicable in a prophylactic vaccine approach for maximum efficacy.

ANTIBODY GENE TRANSFER FOR RESPIRATORY TRACT INFECTIONS

The use of vector-mediated antibody gene transfer has not been limited to just HIV (Table 1). Respiratory syncytial virus (RSV) is a major cause of severe respiratory infection in high-risk populations (such as infants) for which a vaccine is not yet available. Currently, the only way to prevent infection is through the passive
administration of anti-RSV antibodies, such as palivizumab (also known as Synagis, manufactured by MedImmune). This antibody can be administered intramuscularly once each month during the RSV season (winter and spring) to prevent RSV infection. While this treatment is effective, it is costly and limited to high-risk individuals, which are attributes that make it a prime candidate for antibody gene transfer. Instead of repeated monthly injections of the purified antibody, the antibody could be endogenously expressed from a single injection using antibody gene transfer and provide a constant level of protective anti-RSV antibodies in the host.

The study used different vector systems to deliver antibodies against RSV infection (59). They compared the expression and efficacy of a mouse version of palivizumab in a mouse model system when delivered by either a rAAV vector (serotype rh.10) or adenovirus (Ad) vector. Adenovirus vectors have the capacity for high-level gene transfer with rapid and robust transgene expression. However, Ad vectors are highly immunogenic, and transduced cells are quickly cleared by the immune system, resulting in the rapid loss of transgene expression. In contrast, rAAV vectors have very low immunogenicity and can give rise to long-term gene (antibody) expression for potentially the life of the individual. The Ad-palivizumab vector was administered intravenously, with palivizumab detected in the lungs by day 3 postadministration. Following an intranasal RSV challenge 7 days postadministration, the mice showed >5-fold decrease in RSV titers in the lung compared with control animals. Long-term antibody expression and challenge studies were done using the rAAVrh.10-palivizumab vector via intrapleural administration. Palivizumab was detected in the serum of these animals by 8 weeks postadministration that started to peak by week 20. These rAAVrh.10-palivizumab mice were intranasally challenged with RSV at 7 and 21 weeks’ postadministration. They showed 14.3-fold and 10.6-fold lower numbers of RSV PFU in the lungs, indicating that protection against RSV infection can be sustained at least 21 weeks postdelivery of an rAAV vector.

Antibody gene transfer studies using rAAV vectors have also been done to prevent influenza. Although traditional vaccination strategies for influenza are quite effective, they may not be adequate for a possible zoonotic strain that could lead to a pandemic (such as the 2009 H1N1). In this case, the time needed to develop a traditional vaccine may not be rapid enough. The rationale is that vector-mediated antibody gene transfer could quickly deliver a bNAb that is effective against multiple strains of influenza that would provide protection against a pandemic. One study looked at delivering the bNAb antibody FI6 (60) as an immunoadhesin using rAAV serotype 9 via intranasal delivery in mice and ferrets (51). FI6 immunoadhesin expression was detected in the nasal and lung lavage fluids of mice 14 days after vector administration at concentrations ranging from 0.5 to 2.0 μg/ml. Animals challenged as early as 3 days after rAAV9-FI6 administration could be protected. Furthermore, this strategy was able to protect both mice and ferrets from the exposure of lethal doses of various clinical isolates of H5N1 and H1N1. An additional study (61) also demonstrated that rAAV9-FI6 administration showed partial efficacy in mice challenged with the newly emergent avian H7N9, which is believed to be transmitted from poultry to humans.

A separate study used a similar strategy but with intramuscular administration of the rAAV antibody vector in mice (62). They expressed antibodies F10 (63) and CR6261 (64) in an rAAV serotype 8 vector by using the FMDV-2A expression system. Antibody expression levels in the serum reached 200 μg/ml at 5 weeks after intramuscular injection, with levels still around 10 μg/ml out to at least 11 months after a single injection. These treated mice were protected from diverse strains of H1N1 influenza when challenged at either of these time points (5 weeks and 11 months), demonstrating once again the incredible potential for this strategy as a vaccine. The results from both the intranasal (51) and intramuscular (62) routes of vector administration reinforce the flexibility of vector-mediated gene transfer and provide important proof-of-concept studies that could lead to translation into humans.

### TABLE 1: Vector-mediated antibody gene transfer studies

<table>
<thead>
<tr>
<th>Application</th>
<th>Antibody</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV vaccine</td>
<td>SIV immunoadhesins, 4E10, 2G12, 2F5, b12, VRC01, PG9a</td>
<td>Lewis 2002 (47), Johnson 2009 (22), Balazs 2012 (55)</td>
</tr>
<tr>
<td>HIV therapy</td>
<td>10-1074</td>
<td>Horwitz 2013 (56)</td>
</tr>
<tr>
<td>RSV vaccine</td>
<td>Palivizumab</td>
<td>Skaricic 2008 (59)</td>
</tr>
<tr>
<td>Influenza vaccine</td>
<td>FI6, F10, CR6261</td>
<td>Limbers 2013 (61), Balazs 2013 (62)</td>
</tr>
<tr>
<td>Drug addiction</td>
<td>NIC9D9, GNC92H2</td>
<td>Hicks 2012 (63), Rosenberg 2012 (66)</td>
</tr>
<tr>
<td>Cancer therapy</td>
<td>DC101</td>
<td>Fang 2005 (48)</td>
</tr>
</tbody>
</table>

aThe first clinical trial using rAAV vector-mediated PG9 antibody gene transfer began in 2014 as a result of collaboration between The Children’s Hospital of Philadelphia, The International AIDS Vaccine Initiative, and the Division of AIDS (DAIDS) in the National Institute of Allergy and Infectious Diseases.
ANTIBODY GENE TRANSFER FOR DRUG ADDICTION

Perhaps a less conventional use of vector-mediated antibody gene transfer is a potential role in the treatment of drug/substance addiction. Antibodies exist that can bind to these substances in the blood and prevent their transfer to the brain, which leads to their addictive properties. Antibody therapy for addiction would require routine, costly injections, which once again makes this a prime candidate for antibody gene transfer. NIC9D9 is an antinicotine antibody that was delivered intravenously (targeting the liver) to mice by using a rAAVrh.10 vector (65). NIC9D9 antibody was detected in the serum for the length of the 18-week study. Following intravenous nicotine delivery, the rAAV-NIC9D9 mice had 83% of the nicotine bound to the NIC9D9 antibody in serum, which drastically reduced the amount of serum delivered to the brain. Furthermore, these mice had reduced cardiovascular effects compared with control animals. These results indicate that this strategy may hold promise as an effective preventative therapy for nicotine addiction.

Along the same line, GNC92H2 is a mouse monoclonal antibody with high affinity for cocaine. This antibody was also delivered to mice by using the rAAVrh.10 vector via intravenous injection (66). GNC92H2 was detected in the serum for the entire duration of the 24-week study. The GNC92H2 antibody was able to sequester intravenously administered cocaine in the blood, thereby protecting the brain from the effects of cocaine. Furthermore, these mice showed suppressed cocaine-induced hyperactivity derived from weekly cocaine exposure (12 to 17 weeks after rAAVrh.10 vector administration). These findings offer an alternative intervention to cocaine addiction therapy. High-affinity cocaine antibodies could be maintained long term in the serum following a single administration. This strategy could be coupled with traditional behavioral therapies for a combined approach for the treatment of cocaine addiction.

ANTIBODY GENE TRANSFER FOR CANCER

The vector-mediated antibody gene transfer strategy can be expanded for use in noninfectious disease applications where antibodies still play a critical role, such as cancer treatment. In a study by Fang et al. (48), they examined the efficacy of an antitumor antibody to reduce tumor growth in a mouse model system. They used an rAAV8 vector that expressed antibody DC101 by the FMDV-2A system. Antibody DC101 is an antiangiogenic monoclonal antibody that targets vascular endothelial cell growth factor receptor-2 (VEGFR2). Mice given an intravenous injection of rAAV8-DC101 could express high levels (>1 mg/ml) of the antibody in the serum for the length of the 5-month monitoring period. Mice receiving this rAAV vector exhibited shrinkage of tumors and prolonged survival time compared with untreated control animals. These encouraging results set the stage for combining antibody gene transfer technology with the ever increasing number of antibody-based therapies for cancers that include such antibodies as Herceptin and Avastin (Genentech), to name a few.

DRAWBACKS OF rAAV ANTIBODY GENE TRANSFER

Essentially any therapeutic or immunoprophylactic protein can be expressed by using rAAV vector gene transfer, as long as it fits within the vector packaging limit. However, one must be careful that the expressed protein is not immunogenic in the host. However, this is also the same concern for all exogenously (passively) administered proteins, including monoclonal antibodies and other biologics. Most, if not all, of the 25 monoclonal antibodies that have been approved as therapeutics have exhibited some level of immunogenicity (67, 68). Several factors may contribute to immunogenicity including antibody structure, dosing regimen, and the recipient’s genetic background. Also, it remains to be determined if an antibody that was endogenously expressed in the host via gene transfer will be more or less immunogenic than when passively administered as an exogenously produced protein. The big question is what effect would an immune response to the transgene have in the host? In the simplest scenario, as was seen in the non-human primate studies (22), the appearance of anti-antibody responses would limit the vaccine efficiency through loss of transgene expression, with no adverse events observed. Ultimately, at this stage, it is difficult to predict with any certainty which, if any, of the candidates would be immunogenic, and what the consequences would be. Human clinical trials will be the best predictor.

Perhaps of greater concern is the risk that the antibody will bind off-target causing an unanticipated adverse event. Preclinical testing, such as passive administration and Good Laboratory Practice (GLP) human tissue binding studies, can help avert most of these issues. However, if off-target effects occur in vivo, there is currently no efficient method to stop antibody gene expression. As the data shows from animal models,
antibodies are expressed for potentially the life of the host following a single intramuscular administration. A few studies have attempted to regulate gene expression from rAAV gene transfer vectors in mice and monkeys (69, 70, 71), but these schemes are transient and require continuous exogenous drug administration to maintain a constant level of gene expression. Clearly, identifying an efficient method to permanently eliminate antibody gene expression in the host is a top priority if rAAV vector-mediated antibody gene transfer is to become applicable for wide-scale use.

ACKNOWLEDGMENTS
Conflict of interest: We declare no conflicts of interest.

REFERENCES


