Published in final edited form as:

Curr Opin HIV AIDS. 2014 May ; 9(3): 250–256. doi:10.1097/COH.0000000000000056.

Adeno-Associated Virus Delivery of Broadly Neutralizing Antibodies

Bruce C. Schnepp and Philip R. Johnson

The Children's Hospital of Philadelphia and the University of Pennsylvania School of Medicine, Philadelphia, PA 19104, USA

Abstract

Purpose of review—In this review, we will discuss the emerging field of vector mediated antibody gene transfer as an alternative HIV vaccine. This approach is an improvement over classical passive immunization strategies that administer antibodies to the host to provide protection from infection. With vector mediated gene transfer, the antibody *gene* is delivered to the host resulting in long-term endogenous antibody expression from the injected muscle that confers protective immunity.

Recent Findings—A large number of very potent and broadly neutralizing HIV antibodies have recently been isolated and characterized. Vector mediated antibody gene transfer allows one to immediately use these antibodies as a vaccine. Gene transfer studies in both mice and monkeys demonstrate long-term antibody expression in serum from a single injection at concentrations that provide sterilizing immunity.

Summary—Vector mediated antibody gene transfer can rapidly move existing, potent anti-HIV molecules into the clinic. The gene transfer products demonstrate a potency and breadth identical to the original product. This strategy eliminates the need for immunogen design and interaction with the adaptive immune system to generate protection, a strategy that so far has shown little promise.

Keywords

Antibody gene transfer; Vectored immunoprophylaxis; Adeno associated virus; HIV vaccine

Introduction

The need for a safe and effective HIV vaccine is undisputed. In 2012 alone, 1.6 million people died from AIDS related causes, while 2.3 million people were newly infected with HIV [1]. Two HIV-1 Envelope (Env) subunit vaccines tested in Phase 3 clinical trials (Vax003 and Vax004) failed to protect vaccine recipients from infection, and neither diminished viral replication after infection [2,3]. A similar lack of efficacy was also seen from the Step Study, which used recombinant adenovirus vectors (rAd) that expressed multiple HIV-1 proteins [4,5]. The RV144 trial in Thailand tested a canary pox vector

prime/Env protein boost strategy and showed modest efficacy (31%) [6]. 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 [7]. More recently, the HVTN 505 trial was stopped for futility, dealing yet another blow to HIV vaccine efforts [8]. The HVTN 505 trial, which used a DNA prime/rAd boost, showed no difference in HIV-1 infections between those recipients who received the vaccine and those receiving placebo [9]. Vaccine recipients did generate IgG antibodies to Env, however, the majority were non-neutralizing with low reactivity to the V1V2 antigen [9]. These observations underscore the tremendous hurdles that must be overcome to develop an effective HIV vaccine. Foremost is figuring out how to induce antibodies that neutralize a wide array of HIV field isolates. Such antibodies are rare, and until recently, only a handful had been isolated and characterized [10–13]. Over the past few years, more 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 [14–21]. So, what is the best use of the human monoclonals that have been isolated and characterized? Here we will discuss the emerging field of vector-mediated antibody gene transfer, which involves isolating the representative antibody gene and use gene transfer technology to endow a target host with the gene. This strategy is promising in at least 3 respects. First, gene transfer bypasses the adaptive immune response. Success is not dependent on immunogen design or individual immune responses. Second, one can “pre-select” the anti-HIV transgene(s) of interest. In fact, the door is open to molecules other than antibodies that can be engineered to interfere with HIV entry. Finally, multiple steps in the entry pathway can be targeted. For example, 2 separate vectors might be designed to block the gp160-CD4 interaction and block viral fusion.

A new generation of bNAbs

It was initially believed that potent, broadly neutralizing antibodies to HIV were extremely rare and difficult to elicit. In fact for some time, only 4 such antibodies had been identified, known as b12, 2F5, 2G12 and 4E10 [10–13]. These antibodies provided valuable information as to what regions of HIV envelope were potentially sensitive to neutralization, which could aid in better vaccine antigen design. More recently, a large number of new, significantly more potent bNAbs have been identified using improved screening and sequencing techniques. These newer 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 [14–22]. Detailed analyses of these antibodies indicated they are approximately 10–100 fold more potent and have an increased breadth compared to the original 4 isolates. 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) [17,20,23], glycan containing regions in the variable loops (PG9, PG16, PGT121 and PGT128)[15,16], and the membrane-proximal external region (MPER) on gp41 (10E8) [19].

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 bNAbs [24]. For example, highly stable Env trimers have been generated that bind to most of the known neutralizing antibodies, but generally do not bind non-neutralizing antibodies, and could potentially be used as a next generation immunogen [25]. However even with this new wealth of information at hand, generating bNAbs 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 [15,17,18,20,26]. 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 [27,28]. 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.

While induction of bNAbs by various next generation immunization strategies holds promise, the question remains as to the best use the human monoclonals that have already been isolated and characterized. One obvious option is passive immunization. Passive immunization using neutralizing monoclonal antibodies has protected monkeys from simian-human immunodeficiency virus (SHIV) challenge infections [29–35]. More recently, in the study by Moldt et. al. [35], they showed that passively administered PGT121 can mediate sterilizing immunity against SHIV in monkeys at serum concentrations that are significantly lower, suggesting that a protective serum concentration for PGT121 is in the single-digit amount (1.8 µg/mL). While this study demonstrates 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.

Vector-mediated gene transfer to bypass adaptive immune system

Given the difficulties of using the classic concept of passive immunization as a vaccine, 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. Our antibody gene delivery vector of choice is the recombinant adeno-associated virus (rAAV) vector, which is derived from wild-type AAV. AAV is *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 (approximately 80% of humans are seropositive), 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 [36]. rAAV vectors have an established record of high-efficiency gene transfer in a variety of model systems [37,38]. 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 [39].

The 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. 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 rAAV vectors. One method for antibody expression utilizes a two-promoter system whereby the heavy and light chain genes are transcribed independently using two different promoters and polyadenylation signals within the same rAAV vector genome [40]. 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 [41].

Skeletal muscle provides an ideal target for rAAV vector gene transfer. It is easily accessible for injection and can be highly transduced with multiple rAAV serotypes. Following injection, the rAAV vector genome can form stable non-integrating circular episomes that can persist in non-dividing muscle cells [42–44]. Thus, after a single injection, the muscle now serves as a depot to synthesize the bNAbs that are passively distributed to the circulatory system (Figure 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, due to the antibody half-life (approximately 6 days for PGT121 [35]), 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. 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 like hepatitis C virus, malaria, respiratory syncytial virus, and tuberculosis.

Antibody gene transfer in vivo

We first tested the concept of rAAV-mediated antibody gene transfer in animals by using one of the first bNAb isolated, IgG1b12. The human monoclonal IgG1b12 heavy and light chains were cloned independently into an rAAV genome using the two 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 [40]. 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) myofibers produced antibodies; (iii) antibodies were distributed to the circulation; and (iv) such antibodies were biologically active.

Our next objective was to test the gene transfer concept in monkeys in a challenge study. In pilot experiments using the rAAV-IgG1b12 vector, macaques developed antibody responses to the human-derived transgene that effectively shut down expression. To avoid this, we were able to take advantage of native macaque SIV gp120-specific Fab molecular clones that had been derived directly from SIV-infected macaques [45]. When designing the

antibody gene transfer vectors, we chose to express the Fabs as immunoadhesins. Immunoadhesins are chimeric, antibody-like molecules that combine the functional domain of a binding protein like a single chain variable fragment (scFv) or CD4 extracellular domains 1 and 2 (D1D2) with an immunoglobulin constant domain [46] (Figure 2), and have been shown to be effective in disease models including HIV, SIV and influenza [47–49]. A typical immunoadhesin lacks the constant light chain domain and the constant heavy domain 1 (CH1), however, it can be expressed as a single polypeptide from a single promoter, and form dimers through disulfide bonding in the hinge region. Furthermore, immunoadhesins may also be secreted from muscle more efficiently than native antibodies, corresponding to higher serum levels (unpublished observation). While immunoadhesins have many attractive features, 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 to 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 (Figure 2), which could trigger an immune response leading to loss of expression.

For the macaque experiments, we constructed two immunoadhesins derived from two different SIV Fab fragments, as well as a third immunoadhesin containing the rhesus CD4 domains 1 and 2 (D1D2), which was modeled after CD4-Ig fusion proteins [50]. All of the constructs neutralized *in vitro* the proposed SIV challenge stock (SIVmac316), indicating that the immunoadhesins were functioning like the original Fab clones [48]. 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 post injection) and peaked around 6 months with levels reaching 400 µg/mL in some animals [48]. 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 post injection, 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 post injection. Immunoadhesin levels dropped to a stable level of approximately 20 µg/mL, which has persisted for over the last 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 another group performed rAAV vector mediated gene transfer expression/challenge studies, which they called vectored immunoprophylaxis (VIP) [51]. They expressed the native, full antibodies of 2G12, IgG1b12, 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 go on to further show that these rAAV vectors can provide protection following HIV challenge, with antibody serum levels as low as 8.3 µg/mL (VRC01). These encouraging results reinforce the efficacy of this 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 long-term protection is possible.

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. One study to address this used HIV infected humanized mice that received an intravenous injection of a rAAV (serotype 8) vector expressing bNAb 10–1074 [52], which targets the base of the V3 stem of gp120 [53]. These mice maintained a high level of antibody 10–1074 expression 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. Future studies administering rAAV vectors simultaneously expressing multiple bNAbs could possibly reduce or even eliminate the generation of escape mutants.

Potential pitfalls of antibody gene transfer

While this approach holds tremendous promise, there are some factors that may limit its effectiveness. Perhaps the biggest issue is immunogenicity of the antibody, however, this risk is also a concern with passive immunization approaches. At least 22 monoclonal antibodies have been used as therapeutics [54], and all of these have exhibited some level of immunogenicity [55,56]. Several factors may contribute to immunogenicity including structure, dose and recipient's genetic background. Thus, at this stage it is difficult to predict which, if any, of the antibodies would be immunogenic, and what the consequences would be. In our nonhuman primate studies [48], the appearance of anti-antibody responses resulted in loss of transgene expression with no adverse events observed. Another concern is the risk that the antibody will bind to an off-target, host protein causing an adverse event, which has been suggested for HIV antibodies 2F5 and 4E10 [57]. One way to predict this occurrence is to do tissue-binding studies with purified proteins, or expression in mouse models [58]. A final point is shutting off antibody expression if an immune response or off-target binding lead to a detrimental effect. Unlike passive immunization, rAAV vector mediated antibody gene transfer results in long-term antibody expression. Thus, if an adverse event were to occur, there is currently no efficient method to eliminate antibody expression.

Conclusion

This review highlights the features that make vector mediated antibody gene transfer a novel, unconventional, and innovative approach to the substantial challenge of developing an HIV vaccine. In moving this technology into humans, the first clinical trial using rAAV

vector mediated antibody gene transfer is scheduled to begin in 2014 as a result of collaboration between The Children's Hospital of Philadelphia, The International AIDS Vaccine Initiative (IAVI), and DAIDS. The rAAV vector will express the PG9 full antibody using the dual promoter system. Although future human trials would utilize even more potent antibodies such as PGT121, the current PG9 study will provide important information about vector safety, antibody concentration, duration of expression, and neutralization capacity. Ultimately, we feel that antibody gene transfer vectors using molecules that inhibit all steps in HIV entry will create a multilayered blockade against HIV infection and provide a shortcut to an HIV vaccine.

Abbreviations

bNAbs	broadly neutralizing antibodies
FMDV-2A	foot-and-mouth-disease virus 2A domain
ITRs	inverted terminal repeats
MPER	membrane-proximal external region
rAAV	recombinant adeno-associated virus
VIP	vectored immunoprophylaxis

References and recommended reading

1. Getting to Zero. The Global AIDS Epidemic. Press Release. 2012
2. Flynn NM, Forthal DN, Harro CD, et al. Placebo-controlled phase 3 trial of a recombinant glycoprotein 120 vaccine to prevent HIV-1 infection. *J Infect Dis.* 2005; 191:654–665. [PubMed: 15688278]
3. Pitisuttithum P, Gilbert P, Gurwith M, et al. Randomized, double-blind, placebo-controlled efficacy trial of a bivalent recombinant glycoprotein 120 HIV-1 vaccine among injection drug users in Bangkok, Thailand. *J Infect Dis.* 2006; 194:1661–1671. [PubMed: 17109337]
4. Buchbinder SP, Mehrotra DV, Duerr A, et al. Efficacy assessment of a cell-mediated immunity HIV-1 vaccine (the Step Study): a double-blind, randomised, placebo-controlled, test-of-concept trial. *Lancet.* 2008; 372:1881–1893. [PubMed: 19012954]
5. McElrath MJ, De Rosa SC, Moodie Z, et al. HIV-1 vaccine-induced immunity in the test-of-concept Step Study: a case-cohort analysis. *Lancet.* 2008; 372:1894–1905. [PubMed: 19012957]
6. Rerks-Ngarm S, Pitisuttithum P, Nitayaphan S, et al. Vaccination with ALVAC and AIDSVAX to prevent HIV-1 infection in Thailand. *N Engl J Med.* 2009; 361:2209–2220. [PubMed: 19843557]
7. Haynes BF, Gilbert PB, McElrath MJ, et al. Immune-correlates analysis of an HIV-1 vaccine efficacy trial. *N Engl J Med.* 2012; 366:1275–1286. [PubMed: 22475592]
8. Initiative IAV. , editor. IAVIRep. 2013. von Bubnoff A: HVTN 505: "A hard blow".
9. Hammer SM, Sobieszczyk ME, Janes H, et al. Efficacy trial of a DNA/rAd5 HIV-1 preventive vaccine. *N Engl J Med.* 2013; 369:2083–2092. [PubMed: 24099601]
10. Burton DR, Pyati J, Koduri R, et al. Efficient neutralization of primary isolates of HIV-1 by a recombinant human monoclonal antibody. *Science.* 1994; 266:1024–1027. [PubMed: 7973652]
11. Muster T, Steindl F, Purtscher M, et al. A conserved neutralizing epitope on gp41 of human immunodeficiency virus type 1. *J Virol.* 1993; 67:6642–6647. [PubMed: 7692082]
12. Trkola A, Purtscher M, Muster T, et al. Human monoclonal antibody 2G12 defines a distinctive neutralization epitope on the gp120 glycoprotein of human immunodeficiency virus type 1. *J Virol.* 1996; 70:1100–1108. [PubMed: 8551569]

13. Zwick MB, Labrijn AF, Wang M, et al. Broadly neutralizing antibodies targeted to the membrane-proximal external region of human immunodeficiency virus type 1 glycoprotein gp41. *J Virol.* 2001; 75:10892–10905. [PubMed: 11602729]
14. Burton DR, Poignard P, Stanfield RL, et al. Broadly neutralizing antibodies present new prospects to counter highly antigenically diverse viruses. *Science.* 2012; 337:183–186. [PubMed: 22798606] . This article focuses on the the new class of recently isolated potent bNAbs against HIV-1. They discuss how studying their binding characteristics may help to develop new vaccines and drugs.
15. Walker LM, Huber M, Doores KJ, et al. Broad neutralization coverage of HIV by multiple highly potent antibodies. *Nature.* 2011; 477:466–470. [PubMed: 21849977]
16. Walker LM, Phogat SK, Chan-Hui PY, et al. Broad and potent neutralizing antibodies from an African donor reveal a new HIV-1 vaccine target. *Science.* 2009; 326:285–289. [PubMed: 19729618]
17. Wu X, Yang ZY, Li Y, et al. Rational design of envelope identifies broadly neutralizing human monoclonal antibodies to HIV-1. *Science.* 2010; 329:856–861. [PubMed: 20616233]
18. Bonsignori M, Hwang KK, Chen X, et al. Analysis of a clonal lineage of HIV-1 envelope V2/V3 conformational epitope-specific broadly neutralizing antibodies and their inferred unmutated common ancestors. *J Virol.* 2011; 85:9998–10009. [PubMed: 21795340]
19. Huang J, Ofek G, Laub L, et al. Broad and potent neutralization of HIV-1 by a gp41-specific human antibody. *Nature.* 2012; 491:406–412. [PubMed: 23151583]
20. Scheid JF, Mouquet H, Ueberheide B, et al. Sequence and structural convergence of broad and potent HIV antibodies that mimic CD4 binding. *Science.* 2011; 333:1633–1637. [PubMed: 21764753]
21. Zhou T, Georgiev I, Wu X, et al. Structural basis for broad and potent neutralization of HIV-1 by antibody VRC01. *Science.* 2010; 329:811–817. [PubMed: 20616231]
22. Simek MD, Rida W, Priddy FH, et al. Human immunodeficiency virus type 1 elite neutralizers: individuals with broad and potent neutralizing activity identified by using a high-throughput neutralization assay together with an analytical selection algorithm. *J Virol.* 2009; 83:7337–7348. [PubMed: 19439467]
23. Falkowska E, Ramos A, Feng Y, et al. PGV04, an HIV-1 gp120 CD4 binding site antibody, is broad and potent in neutralization but does not induce conformational changes characteristic of CD4. *J Virol.* 2012; 86:4394–4403. [PubMed: 22345481]
24. Kwong PD, Mascola JR, Nabel GJ. Broadly neutralizing antibodies and the search for an HIV-1 vaccine: the end of the beginning. *Nat Rev Immunol.* 2013; 13:693–701. [PubMed: 23969737]
25. Sanders RW, Derking R, Cupo A, et al. A next-generation cleaved, soluble HIV-1 Env Trimer, BG505 SOSIP.664 gp140, expresses multiple epitopes for broadly neutralizing but not non-neutralizing antibodies. *PLoS Pathog.* 2013; 9:e1003618. [PubMed: 24068931]
26. Zhou T, Zhu J, Wu X, et al. Multidonor Analysis Reveals Structural Elements, Genetic Determinants, and Maturation Pathway for HIV-1 Neutralization by VRC01-Class Antibodies. *Immunity.* 2013; 39:245–258. [PubMed: 23911655] . This study examines the B cell lineage of the VRC01 class of antibodies. They determine that a high level of somatic mutations are required to give rise to bNAbs, suggesting that these bNAbs may be difficult to generate with a current vaccine antigen approach.
27. Alam SM, Dennison SM, Aussedat B, et al. Recognition of synthetic glycopeptides by HIV-1 broadly neutralizing antibodies and their unmutated ancestors. *Proc Natl Acad Sci U S A.* 2013; 110:18214–18219. [PubMed: 24145434]
28. Jardine J, Julien JP, Menis S, et al. Rational HIV immunogen design to target specific germline B cell receptors. *Science.* 2013; 340:711–716. [PubMed: 23539181]
29. Baba TW, Liska V, Hofmann-Lehmann R, et al. Human neutralizing monoclonal antibodies of the IgG1 subtype protect against mucosal simian-human immunodeficiency virus infection. *Nat Med.* 2000; 6:200–206. [PubMed: 10655110]
30. Mascola JR, Stiegler G, VanCott TC, et al. Protection of macaques against vaginal transmission of a pathogenic HIV-1/SIV chimeric virus by passive infusion of neutralizing antibodies. *Nat Med.* 2000; 6:207–210. [PubMed: 10655111]

31. Parren PW, Marx PA, Hessel AJ, et al. Antibody protects macaques against vaginal challenge with a pathogenic R5 simian/human immunodeficiency virus at serum levels giving complete neutralization in vitro. *J Virol*. 2001; 75:8340–8347. [PubMed: 11483779]
32. Hessel AJ, Poignard P, Hunter M, et al. Effective, low-titer antibody protection against low-dose repeated mucosal SHIV challenge in macaques. *Nat Med*. 2009; 15:951–954. [PubMed: 19525965]
33. Hessel AJ, Rakasz EG, Poignard P, et al. Broadly neutralizing human anti-HIV antibody 2G12 is effective in protection against mucosal SHIV challenge even at low serum neutralizing titers. *PLoS Pathog*. 2009; 5:e1000433. [PubMed: 19436712]
34. Willey R, Nason MC, Nishimura Y, et al. Neutralizing antibody titers conferring protection to macaques from a simian/human immunodeficiency virus challenge using the TZM-bl assay. *AIDS Res Hum Retroviruses*. 2010; 26:89–98. [PubMed: 20059398]
35. Moldt B, Rakasz EG, Schultz N, et al. Highly potent HIV-specific antibody neutralization in vitro translates into effective protection against mucosal SHIV challenge in vivo. *Proc Natl Acad Sci U S A*. 2012; 109:18921–18925. [PubMed: 23100539] . This study shows that passively administered bNAbs PGT121 can mediate sterilizing immunity against SHIV in monkeys at low serum concentrations. These results provide important evidence that PGT121 antibody gene transfer could be possible.
36. Asokan A. Reengineered AAV vectors: old dog, new tricks. *Discov Med*. 2010; 9:399–403. [PubMed: 20515607]
37. Coura Rdos S, Nardi NB. The state of the art of adeno-associated virus-based vectors in gene therapy. *Virol J*. 2007; 4:99. [PubMed: 17939872]
38. Daya S, Berns KI. Gene therapy using adeno-associated virus vectors. *Clin Microbiol Rev*. 2008; 21:583–593. [PubMed: 18854481]
39. Aalbers CJ, Tak PP, Vervoordeldonk MJ. Advancements in adeno-associated viral gene therapy approaches: exploring a new horizon. *F1000 Med Rep*. 2011; 3:17. [PubMed: 21941595]
40. Lewis AD, Chen R, Montefiori DC, et al. Generation of neutralizing activity against human immunodeficiency virus type 1 in serum by antibody gene transfer. *J Virol*. 2002; 76:8769–8775. [PubMed: 12163597]
41. Fang JM, Qian JJ, Yi SY, et al. Stable antibody expression at therapeutic levels using the 2A peptide. *Nat Biotechnol*. 2005; 23:584–590. [PubMed: 15834403]
42. Nowrouzi A, Penaud-Budloo M, Kaeppl C, et al. Integration frequency and intermolecular recombination of rAAV vectors in non-human primate skeletal muscle and liver. *Mol Ther*. 2012; 20:1177–1186. [PubMed: 22453768] . This study provides important evidence that supports the safety of rAAV vectors. They demonstrate that rAAV vectors predominantly persist as non-integrating, circular extrachromosomal forms in liver and muscle in vivo.
43. Penaud-Budloo M, Le Guiner C, Nowrouzi A, et al. Adeno-associated virus vector genomes persist as episomal chromatin in primate muscle. *J Virol*. 2008; 82:7875–7885. [PubMed: 18524821]
44. Schnepf BC, Clark KR, Klemanski DL, et al. Genetic fate of recombinant adeno-associated virus vector genomes in muscle. *J Virol*. 2003; 77:3495–3504. [PubMed: 12610125]
45. Johnson WE, Sanford H, Schwall L, et al. Assorted mutations in the envelope gene of simian immunodeficiency virus lead to loss of neutralization resistance against antibodies representing a broad spectrum of specificities. *J Virol*. 2003; 77:9993–10003. [PubMed: 12941910]
46. Ashkenazi A, Chamow SM. Immunoadhesins as research tools and therapeutic agents. *Curr Opin Immunol*. 1997; 9:195–200. [PubMed: 9099788]
47. Capon DJ, Chamow SM, Mordenti J, et al. Designing CD4 immunoadhesins for AIDS therapy. *Nature*. 1989; 337:525–531. [PubMed: 2536900]
48. Johnson PR, Schnepf BC, Zhang J, et al. Vector-mediated gene transfer engenders long-lived neutralizing activity and protection against SIV infection in monkeys. *Nat Med*. 2009; 15:901–906. [PubMed: 19448633] . This study was the first to demonstrate that vector mediated antibody gene transfer could provide protection from viral challenge in a nonhuman primate model. They also show evidence of long-term antibody expression as well as the effects of immune reactions to the antibody transgene.

49. Limberis MP, Adam VS, Wong G, et al. Intranasal antibody gene transfer in mice and ferrets elicits broad protection against pandemic influenza. *Sci Transl Med*. 2013; 5:187ra172.
50. Allaway GP, Ryder AM, Beaudry GA, et al. Synergistic inhibition of HIV-1 envelope-mediated cell fusion by CD4-based molecules in combination with antibodies to gp120 or gp41. *AIDS Res Hum Retroviruses*. 1993; 9:581–587. [PubMed: 8369162]
51. Balazs AB, Chen J, Hong CM, et al. Antibody-based protection against HIV infection by vectored immunoprophylaxis. *Nature*. 2012; 481:81–84. [PubMed: 22139420] . These authors used rAAV antibody gene transfer to express bNAbs in humanized mice. The antibodies exhibited long-term expression and were able to protect the mice from HIV infection.
52. Horwitz JA, Halper-Stromberg A, Mouquet H, et al. HIV-1 suppression and durable control by combining single broadly neutralizing antibodies and antiretroviral drugs in humanized mice. *Proc Natl Acad Sci U S A*. 2013; 110:16538–16543. [PubMed: 24043801] . This study used both passive antibody administration and rAAV vector antibody gene transfer as a therapy for HIV infected humanized mice. While treated mice showed reduced viral loads, escape mutants arose indicating the need for simultaneous use of multiple bNAbs.
53. Mouquet H, Scharf L, Euler Z, et al. Complex-type N-glycan recognition by potent broadly neutralizing HIV antibodies. *Proc Natl Acad Sci U S A*. 2012; 109:E3268–E3277. [PubMed: 23115339]
54. Chames P, Van Regenmortel M, Weiss E, et al. Therapeutic antibodies: successes, limitations and hopes for the future. *Br J Pharmacol*. 2009; 157:220–233. [PubMed: 19459844]
55. Keizer RJ, Huitema AD, Schellens JH, et al. Clinical pharmacokinetics of therapeutic monoclonal antibodies. *Clin Pharmacokinet*. 2010; 49:493–507. [PubMed: 20608753]
56. Kessler M, Goldsmith D, Schellekens H. Immunogenicity of biopharmaceuticals. *Nephrol Dial Transplant*. 2006; 21(Suppl 5):v9–v12. [PubMed: 16959792]
57. Yang G, Holl TM, Liu Y, et al. Identification of autoantigens recognized by the 2F5 and 4E10 broadly neutralizing HIV-1 antibodies. *J Exp Med*. 2013; 210:241–256. [PubMed: 23359068] . This study characterizes autoantigens recognized by anti-HIV antibodies, which is important if they are to be used for wide-scale vaccine and therapeutic approaches. They also suggest that immune tolerance to self antigens could impair humoral responses to HIV-1.
58. Doyle-Cooper C, Hudson KE, Cooper AB, et al. Immune Tolerance Negatively Regulates B Cells in Knock-In Mice Expressing Broadly Neutralizing HIV Antibody 4E10. *J Immunol*. 2013; 191:3186–3191. [PubMed: 23940276] . The authors describe the use of mouse models to predict if antibodies will be potentially autoreactive in a host.

Key points

1. Vector mediated antibody gene transfer delivers an antibody gene to the host resulting in endogenous antibody expression from a single intramuscular injection.
2. This strategy bypasses the adaptive immune response and allows immediate use of newly isolated potent bNAbs.
3. Proof-of-concept gene transfer studies in mice and monkeys demonstrate long-term antibody expression and protection from challenge.
4. This strategy can be expanded to include other pathogens that have proven difficult for vaccine development, including hepatitis C virus, influenza, and malaria.

Figure 1. Immunoprophylaxis by antibody gene transfer

Passive immunization involves intravenous delivery of purified antibodies to engender the host with short-lived immunity in serum and mucosa. In contrast, vector mediated antibody gene transfer uses a viral vector to deliver the antibody *gene* to the host via intramuscular injection. The antibody is produced endogenously in the muscle and secreted into the circulatory system and mucosa providing long-term protection from infection.

Figure 2. Antibodies vs. Immunoadhesins

A native antibody is depicted on the left, and two types of immunoadhesins are shown to the right. The immunoadhesin shown in the middle has a single-chain antibody (scFv) joined to an Fc fragment while the immunoadhesin on the right shows domain 1 and domain 2 of CD4 joined to an Fc fragment.