Promise and problems associated with the use of recombinant AAV for the delivery of anti-HIV antibodies

Sebastian P Fuchs1,2 and Ronald C Desrosiers1

Attempts to elicit antibodies with potent neutralizing activity against a broad range of human immunodeficiency virus (HIV) isolates have so far proven unsuccessful. Long-term delivery of monoclonal antibodies (mAbs) with such activity is a creative alternative that circumvents the need for an immune response and has the potential for creating a long-lasting sterilizing barrier against HIV. This approach is made possible by an incredible array of potent broadly neutralizing antibodies (bNAbs) that have been identified over the last several years. Recombinant adeno-associated virus (rAAV) vectors are ideally suited for long-term delivery for a variety of reasons. The only products made from rAAV are derived from the transgenes that are put into it; as long as those products are not viewed as foreign, expression from muscle tissue may continue for decades. Thus, use of rAAV to achieve long-term delivery of anti-HIV mAbs with potent neutralizing activity against a broad range of HIV-1 isolates is emerging as a promising concept for the prevention or treatment of HIV-1 infection in humans. Experiments in mice and monkeys that have demonstrated protective efficacy against AIDS virus infection have raised hopes for the promise of this approach. However, all published experiments in monkeys have encountered unwanted immune responses to the AAV-delivered antibody, and these immune responses appear to limit the levels of delivered antibody that can be achieved. In this review, we highlight the promise of rAAV-mediated antibody delivery for the prevention or treatment of HIV infection in humans, but we also discuss the obstacles that will need to be understood and solved in order for the promise of this approach to be realized.

Molecular Therapy — Methods & Clinical Development (2016) 3, 16068; doi:10.1038/mtm.2016.68; published online 16 November 2016

Since the first reported cases of acquired immunodeficiency syndrome (AIDS) in 1981 (ref. 1) and the identification of the AIDS-causing virus in 1983 (ref. 2), it is estimated that more than 40 million people have died from human immunodeficiency virus (HIV) infection.24 About 35 years have elapsed since the first documented HIV-1 infections and no substantial progress has been made in developing a vaccine that could effectively protect against HIV infection in the vast majority of people.3–6 Similarly, with the single exception of the "Berlin patient,"7–11 eradication of HIV from infected individuals has also not been achievable.12 Although the development of potent antiretroviral drugs has made it possible to vastly extend the life expectancy of HIV-infected individuals, anti-HIV drugs do not cure virus infection.12–20 As of 2014, it was estimated that almost 37 million people were living with HIV globally, with a continuing new infection rate of 2 million per year.21

There are good reasons for believing that development of an effective vaccine against HIV-1 is going to be a very difficult task.22,23 The predicted difficulties have more or less been borne out by vaccine trials in monkeys and in humans.6,8,24 Of the six large-scale, placebo-controlled human efficacy trials of HIV vaccines, three showed no protection against acquisition and two actually showed enhanced acquisition of HIV-1 infection in the vaccine recipient.25–27 Only one of the six vaccine trials, termed RV144 (ref. 38), appeared to show some protective effects against acquisition,39–47 but claims regarding vaccine efficacy have not been straightforward to interpret. Furthermore, none of the six HIV efficacy trials reported a reduction of viral loads in vaccine recipients that became infected.

While attempts to develop improved vaccine strategies continue, many feel that alternate approaches that differ from conventional vaccination may be needed. One such alternate approach is the delivery of anti-HIV monoclonal antibodies (mAbs) by recombinant AAV (rAAV) gene transfer. This technology is independent of the host immune system and AAV-delivered antibodies have the potential to create a long-term sterilizing barrier against HIV. Studies that have employed rAAV vectors to deliver antibodies or antibody-like molecules have shown protective effects against simian immunodeficiency virus (SIV) in monkeys,48,49 simian-human immunodeficiency virus (SHIV) in monkeys50,51 and HIV in humanized mice.12 Although encouraging, efficacy in monkeys was limited by immune responses to the delivered transgene product.48,49,51 AAV-mediated delivery of broadly neutralizing antibodies (bNAbs) also shows promise for inhibiting viral replication and possibly even eradicating infection in HIV-positive individuals. Passive transfer of bNAbs to HIV-infected mice,53–55 SHIV-infected monkeys,56–58 and HIV-infected

1Department of Pathology, Miller School of Medicine, University of Miami, Miami, Florida, USA; 2Institut für Klinische und Molekulare Virologie, Friedrich-Alexander-Universität Erlangen-Nürnberg, Erlangen, Germany. Correspondence: RC Desrosiers (r.desrosiers@med.miami.edu)
Received 28 July 2016; accepted 11 September 2016
The elusive HIV vaccine

Soon after HIV was discovered, the scientific community was optimistic that a vaccine against the AIDS-causing virus could be developed in a timely manner. That belief has unfortunately been shattered. More than 30 years of research have shown that a vaccine against HIV will be much more difficult to develop than the successful vaccines that exist for other pathogens.\(^{5,22,61,62}\) The biggest challenge in the development of an effective HIV vaccine lies in the nature of the virus itself. HIV establishes a continuous presence by the integration of its genetic information into the host genome; it is able to generate and tolerate an enormous degree of genetic variation; and it has evolved a variety of strategies for evading host immune responses.\(^{5,63–70}\) Once HIV establishes the initial infection, it is able to replicate continuously and without relent despite apparently strong humoral and cellular immune responses.\(^{22}\) Factors that contribute to a failed immune control of HIV infection are summarized in Figure 1.\(^{23,65,71–78}\)

Since the first HIV vaccine trial in 1987 (ref. 79), more than 270 trials have followed.\(^{80}\) From these, several vaccine candidates have progressed to a total of six phase I/II or phase III efficacy trials (Table 1).\(^{6,7,21}\) AIDSVAX B/B used in VAX004 (refs. 25–28) and AIDSVAX B/E used in VAX003 (ref. 29) were the first HIV vaccines to enter phase III clinical trials. The vaccine preparations consisted of combinations of HIV recombinant gp120 envelope (env) proteins. As the name implies, AIDSVAX B/B included envelope protein sequences of two clade B isolates (MN, tissue culture derived strain; GNE-8, primary isolate). AIDSVAX B/E included the sequence of a clade B isolate (MN) and the sequence of a clade E isolate (CM244, primary isolate). The goal of the two studies was to test whether the gp120-induced antibodies were capable of preventing acquisition of the virus in high-risk populations. The outcome of the trials showed that the vaccines were not effective at preventing HIV infection; the rates of infection in the vaccinated groups versus the unvaccinated groups were similar. Furthermore, the vaccines had no influence on viral loads, CD4+ T cell counts or progression to AIDS.\(^{25–29}\)

These first vaccine efficacy trials were followed by two very different efficacy trials that were based on viral vectors aimed at eliciting cellular responses against HIV. Vector viruses derived from replication-defective adenovirus serotype 5 (Ad5) were utilized in the STEP study\(^{60–64}\) and the Phambili study;\(^{65,66}\) numbers 3 and 4 of the HIV efficacy trials. The STEP study enrolled HIV-negative Ad5 immune responders who were overrepresented in vaccine recipients who became infected as compared to placebo recipients who became infected. It also included selective sieving of amino acids in genes that were not even included in the vaccine.\(^{60}\) There is no rational explanation for these sieving effect observations. The vaccine did not induce bnAbs, it did not elicit CD8+ cytotoxic T cell (CTL) responses\(^{65,66}\) and viremia was not reduced in individuals that became infected with HIV.\(^{36}\)

HVTN 505 is the sixth efficacy trial. The goal of the vaccine approach used in this trial was to test the efficacy of a DNA prime and Ad5 vector booster immunization in high-risk male or transgender individuals. Because of the results of the STEP and Phambili trials, individuals with high pre-existing immunity to Ad5 were excluded from the study. The HVTN 505 study was prematurely stopped due to futility 24 months after initial enrollment of participants. Therefore, data analysis could only be performed on those individuals who completed the 24-month study visits, about two thirds of the intended enrollment. The vaccine induced cellular and humoral immune responses but failed at preventing HIV infection with no difference in acquisition between the vaccinees and the control group. Also, the vaccine had no influence on viral loads at set point.\(^{37}\)

Vaccine trials in monkeys

Vaccine studies in monkeys using SIV or SHIV have been used to inform and guide the development of vaccine concepts for human clinical trials.\(^{81–86}\) Results from monkey studies can be used to rank
Figure 1  Difficulties associated with immune control of HIV infection. The nature of HIV and the evolution of immune evasion strategies of the virus are responsible for why a HIV vaccine has remained an elusive task. HIV preferentially infects and destroys CD4+ T cells (central mediators of the immune systems), especially in the gut-associated lymphoid tissue (GALT). The virus early establishes a reservoir in latently infected CD4+ T cells by integration of proviral DNA into the host cell genome. Recognition by cytotoxic T cells is further exacerbated by downregulation of MHC class I molecules on the surface of virus-infected cells, which is orchestrated by the viral nef gene. Sensing of the pathogenic intruder by the host innate immune system is counteracted by the HIV-1 genes vif and vpu. Antibody and CD8+ T cell responses are readily escaped by selection of antigenic escape variants facilitated by the high mutation rate of the virus. The error-prone reverse transcriptase causes an enormous sequence diversity of the envelope glycoproteins gp120 and gp41 (up to 35% among clades, 20% within clades, 10% in a single infected individual). An extensive glycan shield on the env trimer shields vulnerable targets on envelope (about 50% of the mass of gp120). Abbreviations: reverse transcriptase (RT); integrase (IN); protease (PR); capsid (CA); matrix (MA); nucleocapsid (NC); long terminal repeat (LTR); group-specific antigen (gag); the pol gene encodes RT, IN and PR; viral infectivity factor (Vif); viral protein R (Vpr); viral protein unique (Vpu); negative regulatory factor (Nef); trans-activator of transcription (tat); regulator of expression of virion proteins (rev); envelope (env) gene encodes the glycoprotein gp160 that is processed into gp120 and gp41.

Table 1  HIV vaccine efficacy trials in humans.

| Trial         | Name of trial       | Clinical trials identifier | Name of vaccine | Vaccine components                                      | Dates       | Population                                      | Estimated enrollment | Efficacy |
|---------------|---------------------|----------------------------|-----------------|---------------------------------------------------------|-------------|--------------------------------------------------|----------------------|----------|
| 1             | VAX 004             | NCT00002441                | AIDSVAX B/B     | gp120 proteins (clade B)                                | 1998–2003   | Adults at risk of sexually transmitted HIV-1 infection | 5,400                | No       |
| 2             | VAX 003             | NCT00006327                | AIDSVAX B/E     | gp120 proteins (clades B and E)                         | 1999–2003   | Intravenous drug users                           | 2,500                | No       |
| 3             | STEP study (HVTN 502) | NCT00095576               | MRKAdS          | HIV-1 gag/pol/nef trivalent Ad5 vector vaccine          | 2004–2007   | Adults at risk of sexually transmitted HIV-1 infection | 3,000                | Enhanced acquisition |
| 4             | Phambili study (HVTN 503) | NCT00413725              | MRKAdS          | HIV-1 gag/pol/nef trivalent Ad5 vector vaccine          | 2007–2007   | Adults at risk of sexually transmitted HIV-1 infection | 800 (of 3,000)       | Enhanced acquisition |
| 5             | RV144               | NCT00223080                | ALVAC-HIV and AIDSVAX B/E | Canarypox vector (HIV-1 env/gag/pro), and gp120 proteins (clades B and E) | 2003–2009   | Adults at risk of sexually transmitted HIV-1 infection | 16,400               | *        |
| 6             | HVTN 505            | NCT00865566                | VRC DNA/rAd5    | DNA plasmid (gag/pol/nef/env), and rAd5 (gag/pol/env)   | 2009–2013   | Men/Transgender at risk of sexually transmitted HIV-1 infection | 2,500                | No       |

*Per-protocol analysis: no significant efficacy; intent-to-treat analysis: no significant efficacy; modified intent-to-treat analysis: 31% efficacy (P = 0.04).
order, or select, the most promising concepts for trials in humans. The SIV strains SIVmac239 (ref. 91) and SIV251 (ref. 92), as well as the SHIV strains SHIV-SF162 (refs. 93,94) and SHIV-AD8 (refs. 95,96) have been preferentially used, but by no means exclusively.22,88,97 The greatest protective efficacy in monkeys has been achieved by using live attenuated strains of SIV, such as those deleted of the nef gene.90,98 Durable protection has been consistently demonstrated against homologous virus challenge in a variety of studies.38–105 However, considerably less protection has been observed by live attenuated strains when the challenge virus was not closely matched in sequence.105–111 This relatively unimpressive level of protection by live attenuated SIV against challenge by a heterologous AIDS virus strain is perhaps analogous to the inability of human infection with wild-type strains of HIV-1 to routinely protect against superinfection by different strains of HIV-1 (refs. 112–116).

The next most impressive degree of protection in monkey vaccine trials has been achieved with a recombinant, replication-competent herpesvirus derived from the simian cytomegalovirus (CMV).117–119 Approximately 50% of vaccinated monkeys have shown a remarkable degree of virological control following stringent SIVmac239 challenge,119 and no detectable signs of virus infection after more than 1 year from the time of infectious exposure.119 The protective effects that were induced by the recombinant CMV vaccine have been associated with broad and unusual effector memory CD8+ T cell responses that recognize non-classical SIV epitopes including those that are restricted by class I antigen E or class II major histo-compatibility complex molecules.120,121 This type of immunogenicity has been found to be a result of the gene-deleted rhesus CMV strain 68–1 that was being used.122,123 However, even the rhesus CMV vaccine conferred no protection against acquisition of the homologous challenge virus and 50% of the vaccinated monkeys showed no protective effects at all.

A variety of vector-based approaches are being examined in monkey testing and some have already advanced to human trials.124–127 A replication-competent vector based on adenovirus type 26 (Ad26) has shown promise in protecting monkeys against stringent SIVmac251 infection126,129 and a version expressing HIV-1 env protein has advanced to phase I clinical trials in humans.130–132 Vectors based on rhesus monkey rhadinovirus, a gamma-2 herpesvirus that has advanced to phase I clinical trials in humans.130–132 Vectors based on rhesus monkey rhadinovirus, a gamma-2 herpesvirus that is closely related to human Kaposi’s sarcoma-associated herpesvirus, are also being used in monkey trials.133,134 Other promising viral vectors that have shown significant protective effects against SIV challenge in monkeys include: modified replicating vaccinia virus Tiantan,135 modified vaccinia Ankara virus,136,137 live recombinant vesicular stomatitis virus, and Semliki Forest virus replicon.138

**BROADLY NEUTRALIZING ANTIBODIES AGAINST HIV**

Following infection with HIV-1, the anti-HIV antibodies that appear over the first 3 to 6 months typically show very strain-specific neutralizing activity, specific for the sequence of the infecting strain of virus.98,139–141 These strain-specific neutralizing antibodies target the most variable regions of the envelope protein, the so-called variable loops, principally V1 and V2 (refs. 139,142). Just as HIV can easily escape a single antiviral drug, HIV variants appear within months that resist neutralization by the early strain-specific neutralizing antibody response.140,141,144,145 While the B cell repertoire evolves and changes in response to the changing virus, it is a race that the B cells do not win.145 On rare occasions, however, antibodies with superior neutralization potency and breadth do emerge.146–148 These potent broadly neutralizing antibodies (bNAbs) emerge on these rare occasions over a prolonged period of years and frequently have unusual structures that allow them to target the concealed, conserved regions of the envelope protein.149,150

Numerous attempts to induce bNAbs by vaccination in humans have not been successful.151 If continually replicating HIV during the long course of infection does not routinely induce bNAbs, it is easy to imagine how difficult it will be to design immunogens to do so. The long-lasting antibody-virus chase continuum that results in these rare and potent bNAbs is consequently associated with unusual characteristics, including: a highly-evolved, high degree of somatic hypermutation (SHM) that can be accompanied by insertions and deletions; very long complementarity determining regions 3 (CDR3s); unusual structures.149,152–156 Despite progress in areas such as reverse or structure-assisted vaccinology, it will remain an enormous challenge to those interested in antigen design and vaccine delivery to overcome these obstacles for developing a truly effective HIV vaccine.72,157–170

Given the difficulties in eliciting antibodies with potent neutralizing activity against a broad range of HIV-1 isolates, considerable interest has emerged in the concept of directly delivering the unusual monoclonal antibodies (mAbs) with the desired properties. More than a dozen distinct, potent bNAbs have now been isolated and characterized from infected humans (Figure 2). They can be roughly categorized into at least five groups: CD4 binding site; manose patch; the membrane-proximal external region on gp41; Apex; the gp120-gp41 interface. The reader is referred to a number of outstanding reviews on the properties of these mAbs.177,178,179,180–182 We may not know how to elicit such antibodies, but we already have this impressive array of potent bNAbs, they are human in origin, and they can be delivered for prevention or therapeutic purposes. The discovery of bNAbs can historically be divided into two phases. In the early 1990s, hybridoma and phage display methods were used to isolate the first bNAbs by adsorbing sera of HIV-infected patients with monomeric gp120 and gp41 antigens. These “first-generation” bNAbs could effectively neutralize clade B viruses at a half-maximal inhibitory concentration range (IC50) of 1 to 10 μg/ml as assessed by in vitro assays, but they were less or not effective against other global HIV isolates.144 Among the first-generation bNAbs were b12, 4E10, 2F5, and 2G12 (refs. 173–182). In the year 2009, the discovery of a second wave of bNAbs began following the development of improved mAb isolation techniques and the screening of larger cohorts of HIV-infected individuals. Selective B cell sorting and B cell capture methods have facilitated the isolation of a spectacular array of potent bNAbs.183,184,185,186,187,188,189,190,191,192,193,194,195,196,197 These “second-generation” bNAbs are broader and two to three orders of magnitude more potent than the earlier generation of neutralizing antibodies.198,199,200 Among the new bNAbs are PG9 and PG16 (ref. 183), 10-1074 (ref. 189), 10E8 (refs. 190,191), 35O22 (ref. 192), 187,188, 10-1074 (ref. 189), 10E8 (refs. 190,191), 35O22 (ref. 192), PGDM1400 (ref. 193), and VRC34.01 (ref. 194).

Passive transfer of first-generation bNAbs has conferred protection against SHIV infection in monkeys; protective effects seen in those experiments could be attributed to both the neutralization activity and the Fc-mediated effector functions of the utilized mAbs.195–203 Consistent with these second-generation bNAbs exhibiting much higher potency in cell culture, they also showed a higher efficacy in vivo as compared to the first-generation bNAbs.204 Either 3BNC117 or 10–1074, which were given to healthy macaques, were capable of completely blocking SHIV acquisition following a single intrarectal challenge with three half-maximal animal infectious doses (AID50), as long as the infused mAb dose was above 5 mg/kg.205 A prevention study in monkeys that was published by the
The mAb PGT121 was tested for its protective efficacy against vaginal SHIV infection. All 10 monkeys that received a PGT121 dose of \( \geq 1 \text{mg/kg} \) showed sterilizing immunity against a single high-dose SHIV-SF162P3 challenge with 300 half-maximal tissue culture infectious doses (TCID50), and three of five monkeys were even protected with a mAb dose of 0.2 mg/kg.206 A modified version of the bnAb VRC01 with mutations in the IgG Fc portion, termed VRC01-LS, exhibited a threefold longer half-life in serum and increased transduction of mucosal tissues than unmodified VRC01 (ref. 207). The improved biochemical properties together with the overall potency of VRC01-LS provided superior protection against single high-dose rectal challenge with the strain SHIV-BaLP4 (refs. 207, 208). Another study utilized the bnAbs VRC01, VRC01-LS, 3BNC117, and 10–1074 to evaluate protective efficacy against SHIV-AD8 acquisition. It was shown that monkeys that received a single mAb by passive transfer required up to 23 weekly low-dose virus challenges to become infected as compared to the control group that became infected after only a median of three challenges.209

Experiments in humanized mice and in monkeys have also demonstrated therapeutic potential of second-generation bnAbs. Infant rhesus macaques were infected with SHIV-SF162P3 by the oral mucosal route and treated as early as 1 day after virus infection with a mix of the bnAbs PGT121 and VRC07. Unlike the untreated animals, the mAb-treated animals were free of virus in plasma and tissue by day 14 and remained free of virus even 6 months after the infectious exposure.56 A separate study employed monkeys that had been chronically infected with SHIV-SF162P3 for 9 months and subsequently infused with mAb cocktails containing b12, 3BNC117, and PGT121 (ref. 57). In the vast majority of animals, plasma viral loads were reduced within 7 days to undetectable levels until a median of 56 days; viremia rebounded when mAb levels decreased to sub-threshold levels. A reduction of cell-associated virus was also noted. In a parallel study, mAb treatment was employed in monkeys 3 months after SHIV-AD8 infection.58 Monotherapy with the bnAbs 3BNC117 or 10–1074 resulted in a rapid decline in viral loads reaching undetectable levels by 4 to 7 days, followed by virus rebound that identified escape mutants to the single mAbs. A single treatment using both mAbs together suppressed viremia for 3 to 5 weeks, and readministration of the mAb combination allowed repeated transient suppression of viremia.

Monotherapy with PG16, NIH45-46s546, PGT128 or 10–1074 resulted in transiently reduced viral loads in humanized mice infected with the strain HIV-1192 (ref. 53). Virus rebound was associated with distinct escape mutations in the envelope gene. However, a single injection of a combination of bnAbs was capable of controlling HIV infection and suppressing viremia to levels below the limit of detection.53 Viral escape from one mAb is somewhat predictable, as the selective immune pressure is not sufficient to inhibit viral replication long-term. Based on in vitro neutralization assays and mathematical prediction models, it has been reported that a combination of three to four potent bnAbs is likely to provide complete or near complete protection against HIV replication.208,211 Another study that utilized a similarly combined passive transfer regimen involving the mAbs PG16, 10–1074, and 3BNC117 confirmed suppressive effects on HIV in humanized mice, which included lowering of free virus in serum, delayed viral rebound after cessation of antiretroviral therapy (ART) and reduction of cell-associated HIV-1 DNA.24

Although ART and multiple bnAbs are able to suppress viremia in infected mice, there are still latent reservoirs of HIV-infected cells that are refractory to those treatments. An approach, called “shock and kill”, that combines ART and inducers of viral transcription has so far failed to eradicate the latent HIV reservoir.212 However, a study in mice showed that a trimix of bnAbs in combination with three inducers was capable of decreasing the HIV reservoir as measured by viral rebound. Interestingly, the data also revealed that suppression of HIV by the passively transferred Abs was dependent on interaction of the IgG Fc with Fc receptors of immune cells suggesting the importance of IgG effector functions.213 Other studies confirmed that Fc receptor-mediated effector functions of bnAbs play a substantial role in inhibiting HIV or SHIV infection.208,213–215 In this context, the antiviral activity of the IgG Fc is directed against both free virus and virus-infected cells. Therefore, the potency or antiviral capacity of an anti-HIV Ab is not only defined by the affinity function of its Fab but also by the effector mechanisms that are mediated by its Fc.208,216
and appeared to be safe at doses up to 14 g of mAb over a 4-week period.\textsuperscript{218–220} Passive administration of 2F5 and 2G12 resulted in a transient reduction of viral loads in five of seven patients; the median decrease of RNA copies/ml in plasma was about 1 log during the treatment phase (day 0–28) while the maximum decrease was 1.5 log.\textsuperscript{221} In a subsequent study, the effect of three bnAbs was tested in a human clinical trial. The goal of the experiment was to examine antibody-mediated suppression of HIV-1 rebound after cessation of ART.\textsuperscript{222} Sequential infusions of the mAbs 2G12, 4E10, and 2F5 to HIV-infected individuals undergoing interruption of ART showed a delay in viral rebound. Passively administered antibodies showed a substantial inhibitory effect in two of eight chronically infected and in all six acutely HIV patients as compared to a control group, and viral rebound was significantly delayed in acutely infected subjects that received mAb therapy versus those that did not receive mAb therapy. The authors also noted that the bnAb 2G12 had the strongest antiviral effect of all three mAbs used, and that the loss of viremia control in 12 of the 14 immunized patients was associated with viral escape from that mAb. No escape mutants were noted for the other two mAbs, 4E10 and 2F5. Another group conducted a similar passive transfer experiment using the same three bnAbs and confirmed the previously obtained results.\textsuperscript{223}

Phase 1 trials have now evaluated safety, pharmacokinetics and functionality of second-generation bnAbs in people as well. The bnAbs VRC01 and 3BNC117 have been among the first of these to prove their potency in humans; results with these mAbs were just published in the year 2015. Twenty-eight healthy volunteers were given intravenous infusions of the mAb VRC01 (ref. 224). VRC01 appeared to be safe and well tolerated; also, no serious adverse events and no dose-related toxicities were noted following the mAb infusions. The mean concentrations over a 28-day period were 35 μg/ml (at 20 mg/kg) and 57 μg/ml (at 40 mg/kg); readministration on day 28 increased the mean concentration in serum to 56–89 μg/ml; the half-life of VRC01 was 15 days. Furthermore, no anti-VRC01 antibody responses were detected in any volunteer at any time. In another human trial, VRC01 was given to HIV-infected individuals. ART-treated and ART-untreated HIV patients received infusions of the VRC01 mAb at a dosage of 1, 5, 20, or 40 mg/kg.\textsuperscript{29} Two mAb infusions, conducted on day 0 and 28, did not reduce the amount of cell-associated viral DNA (also referred to as reservoir) in the ART-treated HIV patients with undetectable viral loads in plasma. However, a single infusion of VRC01 decreased the plasma viral load by 1.1–1.8 log\textsubscript{10} in six of the eight ART-untreated viremic HIV patients. Reduction of viremia was transient and viral loads returned to baseline levels within 56 days after mAb infusion due to waning mAb levels and selection for less sensitive viruses.

Another human trial that employed passive transfer was published in the same year. The bnAb 3BNC117 was tested in 12 healthy and 17 HIV-infected individuals.\textsuperscript{40} A single infusion of the mAb appeared to be safe and well tolerated at all doses tested (1, 3, 10, 30 mg/kg); also, no serious adverse events were noted. The half-life of 3BNC117 was 17 days in healthy volunteers and 9 days in HIV-infected patients. HIV-infected individuals that received lower doses of 3BNC117 showed only small and transient reductions in viral loads followed by a rapid return to baseline levels. However, a single infusion of the mAb at higher doses (10 and 30 mg/kg) reduced the viremia up to 2.5 log\textsubscript{10} in 10 out of 11 subjects, and viral loads remained significantly reduced for 28 days. Emergence of resistant viral strains was variable among the 3BNC117 recipients. Development of increased neutralization resistance was observed in some patients that exhibited escape mutations in the CD4bs and amino acid insertions in the V5 loop of HIV env.

Further experiments were conducted to explore the antiviral capacity of the bnAb 3BNC117 (ref. 225). Suppression of viral load was attributed to clearance of free virus and reduction of virus spread by clearance of virus-infected cells; clearance of virus-infected cells was dependent on Fc-mediated effector functions of 3BNC117. Another study examined the effects of 3BNC117 monotherapy on the host’s antibody responses.\textsuperscript{226} Autologous IgG samples from day 0 and week 24 postinfusion were tested for their capacity to neutralize a panel of HIV-1 pseudoviruses and autologous viruses from day 0 and week 4 postinfusion. It was shown that autologous week 24 IgG, by which time 3BNC117 had already decayed to below detection, had an increased neutralizing activity against weeks 0 and 4 autologous viruses as compared to the neutralizing activity of autologous day 0 IgG. Therefore, patients that received a passive immunization against HIV appeared to develop stronger host antibody responses to their own HIV infection. A separate trial investigated the effects of 3BNC117 on HIV after ART interruption. The results showed that repeated mAb administrations significantly delayed virus rebound as compared to nontreated individuals; but it also revealed that virus rebounded after antibody levels waned, and that use of 3BNC117 alone led to neutralization-resistant escape mutants.\textsuperscript{227}

Although it has been shown that sera from HIV-infected individuals can enhance HIV infection in vitro, there has been no clear evidence that passively transferred antibodies pose a risk to enhancement of HIV infection in vivo.\textsuperscript{228–250} Nonetheless, antibody-dependent enhancement could theoretically represent a problem to passive immunization strategies against HIV. Despite the promise of utilizing bnAbs to prevent or treat HIV infection, reasonable risk assessments will need to be performed for each individual anti-HIV mAb to exclude the chance of increased virus acquisition or increased virus replication following passive transfer to humans.\textsuperscript{229}

### AAV-MEDIATED DELIVERY OF ANTIBODIES AND ANTIBODY-LIKE MOLECULES

With the availability of more than a dozen potent bnAbs, and given developments in antibody engineering that have enhanced biochemical and antiviral properties,\textsuperscript{231–247} it is easy to imagine the potential for the effectiveness of such anti-HIV mAbs in both prevention and therapeutic scenarios. In prevention scenarios, delivery of potent bnAbs could overcome the difficult barriers to trying to induce such antibodies, with the goal of creating a long-term sterilizing barrier to infection. In therapeutic scenarios, the goals would be to greatly reduce viral replication and plasma viral loads, to eliminate the need for continuing antiviral drug therapy, and it would also hope to reduce viral reservoirs over time toward a real cure.

One issue that will need to be addressed, particularly for therapeutic scenarios, is whether some particular combinations of mAbs provide remarkably synergistic levels of protective effects. Do some combinations of potent bnAbs result in a much greater degree of virus neutralizing activity than either alone?\textsuperscript{211} Do some combinations of potent bnAbs make it much more difficult or impossible for the virus to escape the activity of the combination? Does escape from some combinations of potent bnAbs result in virus that is so poorly fit for replication that it can be easily controlled by the host? Does escape from some combinations of potent bnAbs result in virus that is so easily neutralized that it can be well controlled by the host immune responses? These questions can be readily addressed by cell culture and monkey studies.

Maintenance of effective concentrations of mAbs over prolonged periods by passive administration would require repeated, regular infusions over a prolonged period. This does not seem practically...
possible on a large scale for a variety of reasons. First, it would be prohibitively expensive to use on a large scale just for the antibody production, purification and quality control. Second, long-term adherence is certainly likely to be a problem, particularly in many regions of the developing world. Recombinant adeno-associated virus (rAAV) is ideally suited to achieve the goal of long-term delivery (Figure 3). AAV-based gene delivery is considered to be a safe and effective technology.246–257 Numerous studies in monkeys258–261 and people262–275 have shown the successful and safe application of rAAV vectors for the treatment of various genetic diseases. The positive results of clinical trials for lipoprotein lipase deficiency have led to the first gene therapy product to achieve regulatory approval by a governmental health institute.276–279

The only product that is made by rAAV derives from the transgene that was cloned into the vector.254,280–284 Genetically engineered AAV genomes persist in the cell in episomal form and will produce your protein of choice for the lifetime of the cell.285,286 AAV is capable of transducing quiescent cells such as those from skeletal muscle (Figure 4); as long as the transgene product is viewed as self by the host immune system, rAAV-delivered proteins can be secreted for decades from such long-lived cells.287 Several groups have demonstrated the protective efficacy of AAV-delivered antibodies and antibody-like molecules against AIDS virus infection in monkeys and humanized mice.288–291

![Figure 3](image)

**Figure 3** Recombinant adeno-associated virus (rAAV) vectors for the delivery of monoclonal antibodies (mAbs). Wild-type adeno-associated virus (AAV) is a 25 nm small nonenveloped virus that packages a single-stranded DNA genome. The most prominent AAV serotype, AAV2, has a genome size of 4.7 kb and harbors two viral genes (rep and cap) that are flanked by two 145 bp inverted terminal repeats (ITRs). Four Rep proteins (Rep78, Rep68, Rep52, and Rep40) are produced from transcripts using the p5 and p19 promoters, and these proteins are important for viral replication and regulation of AAV gene expression. The virus does not encode a polymerase enzyme and relies on cellular enzymatic activities. Furthermore, AAV relies on the presence of helper viruses such as herpesvirus or adenovirus in order to undergo productive infection (replication, gene expression, and virion production). The cap gene encodes three structural capsid proteins (VP1, VP2, and VP3) from two transcripts using the p40 promoter. For generating recombinant AAV (rAAV), the entire wild-type AAV genome is replaced by a unique transgene cassette (such as for a mAb) flanked by the AAV ITRs, which are the only wild-type sequences remaining. Production of rAAV virions is achieved by triple transfection using the rAAV vector plasmid and two helper plasmids (rAAV), the entire wild-type AAV genome is replaced by a unique transgene cassette (such as for a mAb) flanked by the AAV ITRs, which are the only wild-type sequences remaining. Production of rAAV virions is achieved by triple transfection using the rAAV vector plasmid and two helper plasmids. One helper plasmid encodes the Rep proteins and the other provides the helper viral capsid proteins, which include Rep78 and Rep68. The rAAV vector is produced by transduction of host cells with the rAAV vector and the helper plasmids. The rAAV vector is then harvested from the supernatant of the infected cells.

A pioneering study conducted in rhesus macaques employed AAV-delivered single-chain fragment variable (scFv) immunoadhesins (antibody-like molecules) to protect against SIV infection.46 The genetic material encoding the scFv immunoadhesins 4L6 and 5L7 used in that experiment was small enough to be accommodated by self-complementary AAV (scAAV) vector, a recombinant AAV variant that encapsidates double-stranded DNA.292 The scAAV vector was chosen due to reports of its enhanced transduction capability and performance at achieving higher rates of transgene expression. However, scAAV is limited at packaging longer sequences such as the genetic information of both heavy and light chain sequences of a full-length immunoglobulin G (IgG).293–295 Conventional single-stranded AAV (ssAAV) vector was used to deliver a rhesus CD4 - rhesus IgG fusion construct, termed N4. All three vectors (4L6, 5L7, and N4) had an AAV1 capsid. Following intramuscular injection of the rAAVs, immunoadhesin concentrations in serum reached up to 190 μg/ml by 4 weeks, and levels of immunoadhesins were maintained in some of the scAAV recipients above 200 μg/ml through 12 months. The nine AAV-immunized monkeys and two groups of control monkeys were challenged with a high dose of the strain SIVmac316 at 4 weeks following the AAV gene transfer. While all six control monkeys became infected by the SIV challenge, six of the nine AAV-immunized monkeys that maintained reasonable levels of immunoadhesins showed sterile protection against SIV exposure.
Following intramuscular inoculation, rAAV binds to a F2A peptide technology as previously described.297,298 Two scAAV vectors or a single ssAAV vector, encapsidated by AAV1, were injected intramuscularly into 12 rhesus macaques and levels of AAV-delivered Abs were measured over time.46 The concentration of the SIV-specific antibodies in serum ranged from 1 to 270 μg/ml through 44 weeks, regardless which AAV vector delivery system was being used. However, the conversion to authentic IgG sequences did not prevent the emergence of anti-antibody responses and this emergence limited the concentration of the SIV-specific antibodies that could be achieved. Nonetheless, we progressed with a challenge phase and conducted a repeated low dose challenge regimen using the neutralization-resistant strain SIVmac239. Although 4L6 and 5L7 IgGs showed no neutralizing activity in vitro, they exerted antiviral effects against highly pathogenic SIVmac239 challenges in vivo as assessed by the significant delay and reduction of viremia in plasma.29

Another study conducted in monkeys utilized AAV-antibody gene transfer to protect against SHIV infection.31 Rhesus macaques were injected intramuscularly with a ssAAV8 vector expressing the potent bnAb VRC07. In four of four test animals, the serum concentration of the AAV-delivered antibody reached 8 μg/ml by week 4 and plummeted to undetectable levels by week 9. This was apparently due to a vigorous anti-VRC07 antibody response despite extensive attempts to make the VRC07 mAb as “rhesuzized” as possible. A second group of monkeys then received the immunosuppressive agent cyclosporine A (CsA) prior to the AAV-antibody gene transfer. Although levels of delivered VRC07 reached serum concentrations as high as 66 μg/ml by week 3, anti-VRC07 antibody responses lowered the AAV-delivered antibody during and especially after immunosuppression. Following challenge with the strain SHIV-BaLP4, significantly more control monkeys became infected as compared to the AAV-antibody group.

One of the most potent and broad molecules capable of inhibiting AIDS virus entry is the antibody-like construct eCD4-Ig.50 This molecule is composed of the outer two domains of CD4 (entry receptor of the AIDS virus), the Fc portion of IgG and a CCR5 (entry coreceptor of the AIDS virus) mimetic peptide that is derived from the HIV-specific antibody E51 (refs. 299–302). While some significant number of HIV-1 isolates are resistant to neutralization by potent bnAbs, eCD4-Ig has neutralized 100% of the tested neutralization-resistant strains. Furthermore, eCD4-Ig has been shown to potently neutralize HIV type 2 and the neutralization-resistant strain SIVmac239. Following AAV1 gene transfer to monkeys, levels of the antibody-like molecule eCD4-Ig ranged between 17 and 77 μg/ml by week 30. The four AAV-immunized macaques and four control macaques were then repeatedly challenged with progressively increasing doses of SHIV-AD8. Complete protection was demonstrated in the AAV-immunized animals, while all control animals became infected following the last challenge that utilized 4 AID50. Interestingly, anti-eCD4-Ig host antibody responses were low or absent, while AAV-transferred potent bnAbs used in that study elicited moderate to strong anti-antibody responses.50

AAV-antibody gene transfer has also been used in humanized mice experiments. Notably, AAV-delivered bnAbs b12, VRC01 and 10–1074 have demonstrated protective effects against HIV acquisition and durable control of HIV in a therapeutic setting.52,54,291 Although mouse experiments can demonstrate whether potent bnAbs have the ability to block or inhibit HIV infection in vivo, the humanized mouse model has certain limitations and may fall short when evaluating HIV pathogenesis, as well as safety and immunogenicity of AAV-delivered antibodies. Since virus challenge experiments are performed in immunocompromised mice that have been engrafted with human cells, it is difficult to translate results to immunocompetent monkeys or humans.88

One trial is currently ongoing to evaluate safety, deliverability, and potential efficacy of rAAV-delivered potent bnAbs: PG9 in uninfected human volunteers in England.127

**OBSTACLES FOR EFFICIENT AAV-ANTIBODY DELIVERY**

Several features of the rAAV vector delivery system may serve to limit the effectiveness with which the desired protein can be expressed. As with any virus, AAV can be recognized as foreign by the host immune system.301–303 While rAAV vector does not directly express any wild-type AAV proteins, rAAV on its own may trigger
innate immune responses.307 Furthermore, pre-existing cellular308 and humoral309 immunity to wild-type AAV may significantly limit the ability of the rAAV to “take” in the host.310

There are 12 AAV serotypes and more than 100 variants (serovars) as specified by phylogenetic analyses.252,311–313 A number of studies have reported that individual AAVs can be sensed by pattern recognition receptors (PRRs), which can lead to upregulation of host defense genes and the production of proinflammatory cytokines and chemokines. This in turn will activate cells of the innate immune system and may amplify the inflammatory signal by initiating adaptive immune responses. Toll-like-receptors (TLR) such as TLR-2 and TLR-9 have been shown to be involved in innate immune responses to AAV by recognizing the AAV capsid and the AAV genome, respectively.314–318

The prevalence of anti-AAV capsid IgG in the human population varies by AAV serotype; e.g., up to 72% of people are sero-positive for AAV2, 67% for AAV1 and 38% for AAV8. Also, antibodies against one serotype may cross-react against another serotype depending on how similar their capsid sequences are.319–321 Neutralizing antibodies in serum at titers of more than 1:5 may already be sufficient to capture intravenously injected virus particles, and by so doing severely reduce transduction by rAAV.322–324 Furthermore, several groups have explored the ability of AAV re-administration to muscle with moderate or no success when the exact same serotype was used; only in the event of immunosuppressive or immunomodulatory intervention was it possible to achieve effective uptake of the same AAV serotype.328–330 Similarly, presence and activation of AAV capsid-specific memory CD8+ T cells can eliminate cells that have taken up rAAV particles.311 Human trials that have employed rAAV to provide functional protein to individuals with hereditary disorder have reported anti-capsid responses to AAV2, AAV8 and AAV1 (refs. 263,264,269). AAV gene transfer may also elicit immune responses against the rAAV-delivered transgene product if the host has never seen that specific protein; in that context, the magnitude of the response is dependent on the degree the endogenous gene is different from the delivered gene, in particular whether the host protein may be truncated or missing entirely.322–330 Human trials have reported transgene-specific cellular responses against rAAV-delivered α1-antitrypsin (AAT), mini-dystrophin protein and coagulation factor IX (F9).263,269,337–339 Furthermore, the magnitude and frequency of immune responses to rAAV vector and delivered transgene product is influenced by several other factors including the AAV serotype or variant that is being used, rAAV tropism for antigen-presenting cells (APCs), the rAAV dose and the route of rAAV administration.330–350

When considering use of rAAV for delivery of mAbs, the first inclination is to assume that human antibodies are natural protein products of humans and should therefore not be viewed as foreign. However, things may not be that simple. The human B cell repertoire can create an enormous number of different antibodies with enormous sequence variation.165,351,352 A particular antibody being made by one individual will not likely ever have been seen by another individual and will likely be less tolerated by the other individual. Furthermore, any particular antibody being made by an individual must have been accepted by a complex checkpoint system during B cell development within that host.353–358 These considerations are further exaggerated by the highly evolved, highly mutated nature of the potential bnAbs one wants to deliver for the prevention or treatment of HIV infection.150 bnAbs have undergone extensive SHM in their variable domains, which allows them to retain enhanced antiviral potency and breadth, but this may also be associated with some self-reactivity and with immunogenicity.

CDR-sequence containing regions of variable domains of IgGs (idiotype variation) may contain CD4+ T cell epitopes that induce unwanted immune responses in the mAb recipient.361,362 Other properties of mAbs may also contribute to their immunogenicity in the recipient host: allotypic variation, misfolding, aggregation and differences in glycosylation.363,364 Immune responses following passive transfer to humans have been reported for a number of therapeutic mAbs.365–368 Although species-specific antibodies have shown to have less immunogenic potential, immune responses to mAbs in humans have occurred independently of the nature of the transferred mAb: murine versus humanized versus fully human.365,369–371

There have been five monkey trials to date where rAAV has been used to deliver antibodies or antibody-like molecules against HIV or SIV. The pioneering study by Johnson et al.48 utilized rAAV1 to deliver the antibody-like molecules (immunoadhesins) 4L6, 5L7, and N4 as prophylaxis against SIV challenge. In contrast to the heavy and light chain coding sequences of a full-length mAb, the coding sequence of an immunoadhesin is small enough to be accommodated by scAAV; this vector type was being used for 4L6 and 5L7. Three of nine rhesus monkeys developed anti-immunoadhesin responses, and these three monkeys were not protected from SIV infection. Although 4L6 and 5L7 are composed of fully rhesus-derived sequences, humoral responses targeted these sequences. The authors found that reactivity was confined to the variable domains of these two immunoadhesins. Humoral responses were also measured against the rhesus CD4 moiety of N4, albeit modest. It is worth noting that 4L6 and 5L7 are extremely hypermutated and bear very long CDR3 sequences. Also, sequences of heavy and light chains were obtained by phage display, which might not resemble a natural pairing of these chains. The artificial fusion of variable light (VL) and variable heavy (VH) domains, as well as CD4 with the IgG Fc could have potentially created conformational epitopes that could be immunogenic.372 Our group converted those immunoadhesin sequences to authentic IgG molecules to potentially avoid any unnatural structures.376 However, Fuchs et al.39 and Martinez-Navio et al.39 found that full-length IgG versions of 4L6 and 5L7 did not prevent anti-antibody responses. Six out of six monkeys that received 4L6 IgG1 and three out of six that received 5L7 IgG1 generated anti-antibody responses. Both heavy and light chain variable regions were targeted including measured reactivity to the heavy chain CDR3 (ref. 359).

Our group has also delivered rhesusized versions of anti-HIV bnAbs (1NC9, 8ANC195, 3BNC117, 10–1074, and 10E8) to monkeys; anti-antibody responses were readily detected against all AAV-delivered antibodies in all eight animals.390 The levels of delivered mAbs were driven to below detection in all animals for all antibodies for which specific detection methods were available. Immunogenicity of the tested anti-HIV bnAbs correlated significantly with the degree of sequence divergence from germline.391 In another study, Saunders et al.39 delivered the HIV-specific bnAb VIRCO7 using AAV8. Four of four monkeys elicited anti-antibody responses to the mAb, and these unwanted anti-antibody responses resulted in a loss of transgene product in all animals by 9 weeks following rAAV administration.39 It is worth noting that the vigorous anti-antibody responses were mounted against VIRCO7, despite extensive efforts to “rhesusize” the mAb as much as possible. The bnAb VIRCO7 was created by pairing the light chain of the bnAb VIRCO1 with a heavy chain isolated from a B cell clone of the VIRCO1 lineage.392 This unnatural pairing of heavy and light chains, along with the 14% SHM rate of VIRCO1 (ref. 167) (full-length antibody sequence as compared to full-length germline sequence of an immunoadhesin is small enough to be accommodated by scAAV; this vector type was being used for 4L6 and 5L7. Three of nine rhesus monkeys developed anti-immunoadhesin responses, and these three monkeys were not protected from SIV infection. Although 4L6 and 5L7 are composed of fully rhesus-derived sequences, humoral responses targeted these sequences. The authors found that reactivity was confined to the variable domains of these two immunoadhesins. Humoral responses were also measured against the rhesus CD4 moiety of N4, albeit modest. It is worth noting that 4L6 and 5L7 are extremely hypermutated and bear very long CDR3 sequences. Also, sequences of heavy and light chains were obtained by phage display, which might not resemble a natural pairing of these chains. The artificial fusion of variable light (VL) and variable heavy (VH) domains, as well as CD4 with the IgG Fc could have potentially created conformational epitopes that could be immunogenic.372 Our group converted those immunoadhesin sequences to authentic IgG molecules to potentially avoid any unnatural structures.376 However, Fuchs et al.39 and Martinez-Navio et al.39 found that full-length IgG versions of 4L6 and 5L7 did not prevent anti-antibody responses. Six out of six monkeys that received 4L6 IgG1 and three out of six that received 5L7 IgG1 generated anti-antibody responses. Both heavy and light chain variable regions were targeted including measured reactivity to the heavy chain CDR3 (ref. 359).

Our group has also delivered rhesusized versions of anti-HIV bnAbs (1NC9, 8ANC195, 3BNC117, 10–1074, and 10E8) to monkeys; anti-antibody responses were readily detected against all AAV-delivered antibodies in all eight animals.390 The levels of delivered mAbs were driven to below detection in all animals for all antibodies for which specific detection methods were available. Immunogenicity of the tested anti-HIV bnAbs correlated significantly with the degree of sequence divergence from germline.391 In another study, Saunders et al.39 delivered the HIV-specific bnAb VIRCO7 using AAV8. Four of four monkeys elicited anti-antibody responses to the mAb, and these unwanted anti-antibody responses resulted in a loss of transgene product in all animals by 9 weeks following rAAV administration.39 It is worth noting that the vigorous anti-antibody responses were mounted against VIRCO7, despite extensive efforts to “rhesusize” the mAb as much as possible. The bnAb VIRCO7 was created by pairing the light chain of the bnAb VIRCO1 with a heavy chain isolated from a B cell clone of the VIRCO1 lineage.392 This unnatural pairing of heavy and light chains, along with the 14% SHM rate of VIRCO1 (ref. 167) (full-length antibody sequence as compared to full-length germline...
sequence) and the further mutated VRC07 heavy chain sequence, may have contributed to the immunogenicity of VRC07. In a second group of animals, use of CsA did not prevent anti-VRC07 antibody responses, but humoral responses were blunted in three of six monkeys by that immunosuppressive intervention, and those three monkeys maintained measurable mAb levels through 16 weeks.51

Gardner et al.50 delivered the broad and potent anti-HIV entry inhibitor eCD4-Ig by AAV1 to monkeys. While two of four monkeys had a weak anti-eCD4-Ig response, the other two showed no detectable anti-inhibitor reactivity. Comparably modest anti-inhibitor responses have also been observed with N4 (ref. 48). Since rhesus CD4 and rhesus IgG Fc are self proteins to rhesus monkeys, no considerable humoral responses were elicited.50 Furthermore, no reactivity was raised against the CCR5 mimetic peptide, a CDR3-derived peptide299,301 that was artificially fused to the IgG Fc.50 Apparently, the amino acid sequence and the arrangement of the CCR5 mimetic peptide have not presented a major immunogenic stimulus in monkeys. The same group also tested the immunogenicity of the AAV-delivered bnAbs 3BNC117, NIH45-45, 10–1074, and PGT121 in monkeys.50 The bnAbs elicited significantly higher anti-antibody responses as compared to eCD4-Ig. The rate of SHM among those four bnAbs is relatively high: 3BNC117 (36.9%), NIH45-46 (44%), 10–1074 (24%), and PGT121 (21.2%).167

The inherent nature of an anti-HIV bnAb may be sufficient to elicit immune responses in the recipient host since the recipient likely never would have generated or experienced the specific variable domains. Human mAbs used therapeutically have been shown to elicit immune responses in a substantial fraction of humans following passive transfer.365,366,368 To our knowledge, no side-by-side comparison has been conducted that evaluates the immunogenicity of a mAb when administered passively versus by AAV gene transfer. The anti-HIV bnAb VRC01 did not appear to elicit anti-VRC01 antibody responses in humans following one or two administrations.224 A simianized version of VRC01 elicited anti-VRC01 antibodies in two of eight macaques following four passive administrations.208 The simianized mAb VRC07 elicited robust anti-VRC07 antibody responses in four of four monkeys when delivered by rAAV51, similar to the experience of Martinez-Navio et al. with a variety of AAV-delivered rhesus and rhesusized human mAbs.359 Again, the anti-anti responses to the AAV-delivered mAbs were directed principally or exclusively to the variable domains, i.e., they were anti-idiotypic in nature.31,359

A number of studies have explored ways of reducing immune responses toward a variety of AAV-delivered gene products. The use of immunosuppressive agents such as CsA has shown partial success at reducing immune responses and facilitating transgene expression in monkeys.51 Temporary inhibition of CD4+ T cells has shown to be effective at preventing immune responses against AAV-mediated gene delivery, particularly in the context of AAV readministration in mice.369 A single patient case report showed that combined use of intravenous immunoglobulin (IVIG), B cell ablation and a corticosteroid has allowed for successful AAV-mediated gene transfer in the absence of immune responses towards AAV capsid and the delivered transgene product.375 Passive transfer of a large dose of mAb prior to recombinant AAV administration may circumvent the problem of “inverse dose-immunogenicity relationship”365,375. If readministration of rAAV is desired, the second AAV inoculation could employ a different serotype than the one used in the primary inoculation. Also, the use of engineered AAV capsids may help at minimizing host immune responses; AAV capsid mutations that involve Tyr, Lys, Ser, and Thr residues have shown to improve AAV transduction, and such capsid mutations could allow efficient AAV gene transfer at a lower AAV dose while potentially reducing the sensing by the innate immune system.345,376–378 Use of specific microRNA binding sites (miRNAb) within the rAAV genome may prevent transgene expression in professional antigen presenting cells (APCs) and thus inhibit elicitation of immune responses.379,380 Liver-directed AAV gene transfer may accomplish induction of tolerance toward any mAb. Expression of transgene products in liver tissue has been demonstrated to be tolerogenic by mechanisms that include but are not limited to induction of regulatory T cells (Tregs).381–386

SUMMARY

Wild-type AAV has never been associated with the cause of any known diseases in humans, and recombinant AAV has demonstrated its overall efficacy and safety in more than 120 clinical trials, with transient tissue inflammation as the most severe side effect.256,387 Given the need to explore unconventional approaches against HIV, AAV-mediated delivery of potent anti-HIV bnAbs represents a promising approach for the prevention and treatment of HIV infection. Trials in monkeys have demonstrated significant efficacy of rAAV-delivered antibodies and antibody-like molecules for prevention of AIDS virus infection. Nonetheless, despite the safe and effective application that has been attributed to AAV-mediated gene transfer, immune responses to AAV-delivered antibodies remain the most significant impediment that will limit the effectiveness of this approach. This impediment needs to be better understood and overcome for the promise of the AAV-antibody approach to be effectively realized in people.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

The authors would like to thank J.M. Martinez-Navio for critically reading the manuscript and helpful advice.

REFERENCES

1. Gottlieb, MS, Schroff, R, Schanker, HM, Weisman, JD, Fan, PT, Wolf, RA et al. (1981). Pneumocystis carinii pneumonia and mucosal candidiasis in previously healthy homosexual men: evidence of a new acquired cellular immunodeficiency. N Engl J Med 305: 1425–1431.
2. Barré-Sinoussi, F, Chermann, JC, Rey, F, Hugues, MT, Chamaret, S, Gruest, J et al. (1983). Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS). Science 220: 868–871.
3. UNAIDS (2000). REPORT on the global HIV/AIDS epidemic. http://www.unaids.org/sites/default/files/media_asset/UNAIDS_Global_Report_2013_en_1.pdf.
4. UNAIDS (2013). Global report: UNAIDS report on the global AIDS epidemic. http://www.unaids.org/sites/default/files/media_asset/UNAIDS_Global_Report_2013_en_1.pdf.
5. Barré-Sinoussi, F, Ross, AL and Delfraissy, JF (2013). Past, present and future: 30 years of HIV research. Nat Rev Microbiol 11: 877–883.
6. Esparza, J (2013). A brief history of the global effort to develop a preventive HIV vaccine. Vaccine 31: 3502–3518.
7. Barry, SM, Mena Lora, AJ, and Novak, RM (2014). Trial, error, and breakthrough: a review of HIV vaccine development. J AIDS Clin Res 6: 359.
8. Girard, MP, Osmanov, S, Assoussi, OM and Kieny, MP (2011). Human immunodeficiency virus (HIV) immunopathogenesis and vaccine development: a review. Vaccine 29: 6191–6218.
9. Hütter, G, Nowak, D, Messner, M, Ganepola, S, Müßig, A, Allers, K et al. (2009). Long-term control of HIV by CR5 Delta32/Delta32 stem-cell transplantation. N Engl J Med 360: 692–698.
10. Allers, K, Hütter, G, Hofmann, J, Loddenkemper, C, Rieger, K, Thiel, E et al. (2011). Evidence for the cure of HIV infection by CR5 Delta32/Delta32 stem cell transplantation. Blood 117: 2791–2799.
11. Yukl, SA, Boritz, E, Busch, M, Bentzen, C, Chun, TW, Douek, D et al. (2013). Challenges in detecting HIV persistence during potentially curative interventions: a study of the Berlin patient. PLoS Pathog 9: e1003347.
112. Siliciano, JD and Siliciano, RF (2016). Recent developments in the effort to cure HIV infection: going beyond N = 1. J Clin Investig 126: 409–14.
13. Gunthard, HF, Abeg, JA, Eron, JJ, Hoy, JF, Tenenti, A, Benson, CA et al.; International Antiviral Society-USA Panel. (2014). Antiretroviral treatment of adult HIV infection: 2014 recommendations of the International Antiviral Society-USA Panel. JAMA 312: 410–425.
14. Nakagawa, F, May, M and Phillips, A (2013). Life expectancy living with HIV: recent estimates and future implications. Curr Opin Infect Dis 26: 17–25.
15. Siliciano, JD, Kajdas, J, Finzi, D, Quinn, TC, Chakvichad, K, Margolick, JB et al. (2003). Long-term follow-up studies confirm the stability of the latent reservoir for HIV-1 in resting CD4+ T cells. Nat Med 9: 727–728.
16. Strain, MC, Gunthard, HF, Hafv, DV, Ignacio, CC, Smith, DM, Leigh-Brown, AJ et al. (2003). Heterogeneous clearance rates of long-lived lymphocytes infected with HIV: intrinsic stability predicts lifelong persistence. Proc Natl Acad Sci USA 100: 4819–4824.
17. Finzi, D, Hermankova, M, Pierson, T, Carruth, LM, Buck, C, Chaisson, RE et al. (1997). Identification of a reservoir for HIV-1 in patients on highly active antiretroviral therapy. Science 278: 1295–1300.
18. Wong, JK, Hezareh, M, Gunthard, HF, Hafv, DV, Ignacio, CC, Spina, CA et al. (1997). Recovery of replication-competent HIV despite prolonged suppression of plasma viremia. Science 278: 1291–1295.
19. Chun, TW, Stuyver, L, Mizell, SB, Ehler, LA, Mican, JA, Baseler, M et al. (1997). Presence of an inducible HIV-1 latent reservoir during highly active antiretroviral therapy. Proc Natl Acad Sci USA 94: 13112–13117.
20. Finzi, D, Blankson, J, Siliciano, JD, Margolick, JB, Chakvichad, K, Pierson, T et al. (1999). Latent infection of CD4+ T cells provides a mechanism for lifelong persistence of HIV-1, even in patients on effective combination therapy. Nat Med 5: 512–517.
21. UNAIDS (2015). AIDS by the numbers. http://www.unaids.org/sites/default/files/media_asset/AIDS_by_the_numbers_2015_en.pdf.
22. Desrosiers, RC (2004). Prospects for an AIDS vaccine. Nat Med 10: 221–223.
23. Desrosiers, RC (1999). Strategies used by human immunodeficiency virus that allow persistent viral replication. Nat Med 5: 733–725.
24. Lifson, JD and Hairwood, NL (2012). Lessons in human primate models for AIDS vaccine research: from minifields to milestones. Cold Spring Harb Perspect Med 2: a007310.
25. Flynn, NM, Fothal, DL, Harro, CD, Judson, FN, Mayer, KH and Para, HF, rgp120 HIV Vaccine Study Group (2005). Placebo-controlled phase 3 trial of a recombinant glycoprotein 120 vaccine to prevent HIV-1 infection. J Infect Dis 191: 654–665.
26. Jones, NG, DeCamp, A, Gilbert, P, Peterson, ML, Gurwitz, M and Cao, H (2009). AIDSVAX immunization induces HIV-specific CCR5+ T cells in high-risk, HIV-negative volunteers who subsequently acquire HIV infection. Vaccine 27: 1136–1140.
27. Gilbert, PB, Peterson, ML, Follmann, D, Hudgens, MG, Francis, DP, Gurwitz, M et al. (2005). Correlation between immunologic responses to a recombinant glycoprotein 120 vaccine and incidence of HIV-1 infection in a phase 3 HIV-1 preventive vaccine trial. J Infect Dis 191: 666–677.
28. Gilbert, PB, Ackers, ML, Berman, PW, Francis, DP, Popovich, V, Hu, DJ et al. (2005). HIV-1 vireologic and immunologic progression and initiation of antiretroviral therapy among HIV-1-infected subjects in a trial of the efficacy of recombinant glycoprotein 120 vaccine. J Infect Dis 192: 974–983.
29. Pitsuttithum, P, Gilbert, P, Gurwitz, M, Heyward, W, Martin, M, van Griensven, F et al. (2006). Bangkok Vaccine Evaluation Group. (2006). Randomized, double-blind, placebo-controlled efficacy trial of a bivalent recombinant glycoprotein 120 HIV vaccine among injection drug users in Bangkok, Thailand. J Infect Dis 194: 1661–1671.
30. Buchbinder, SP, Mehotra, DV, Duerr, A, Fitzgerald, DW, Mogg, R, Li, D et al; Step Study Protocol Team. (2008). Efficacy assessment of a cell-mediated immunogenic HIV-1 vaccine (the Step Study): a double-blind, randomised, placebo-controlled, test-of-concept trial. Lancet 372: 1881–1893.
31. McElrath, MJ, De Rosa, SC, Moodie, Z, Dubey, S, Kierstead, L, Jones, H et al; Step Study Protocol Team. (2008). HIV-1 vaccine-induced immunity in the test-of-concept Step Study: a case-cohort analysis. Lancet 372: 1894–1905.
32. Duerr, A, Huang, Y, Buchbinder, S, Coombs, RW, Sanchez, J, del Rio, C et al; Step/HVTN 503 Study Team. (2012). Extended follow-up confirms early vaccine-enhanced risk of HIV acquisition and demonstrates waning effect over time among participants in a randomized trial of recombinant adenovirus HIV vaccine (Step Study). J Infect Dis 206: 258–266.
33. Koblin, BA, Mayer, KH, Noonan, E, Wang, CY, Marmor, M, Sanchez, J et al. (2012). Sexual risk behaviors, circumcision status, and preexisting immunity to adenovirus type 5 among men who have sex with men participating in a randomized HIV-1 vaccine efficacy trial: study J Acquir Immune Defic Syndr 60: 405–413.
34. Cheng, CWang, L, Gall, JG, Nason, M, Schwartz, RM, McElrath, MJ et al. (2012). Decreased pre-existing Ad5 capsid and Ad35 neutralizing antibodies increase HIV-1 infection risk in the Step trial independent of vaccination. PLoS one 7: e33969.
35. Gray, GE, Allen, M, Moodie, Z, Churchyard, G, Bekker, LG, Nchabeleng, M et al; HVTN 503/P303 study team. (2011). Safety and efficacy of the HVTN 503/P303 study of a clade B-based HIV-1 vaccine in South Africa: a double-blind, randomised, placebo-controlled test-of-concept phase 2b study. Lancet Infect Dis 11: 507–515.
117. Hansen, SG, Vierlev, C, Whizin, N, Coyne-Johnson, L, Siess, DC, Drummond, DD et al. (2009). Effecter memory T cell responses are associated with protection of rhesus monkeys from mucosal simian immunodeficiency virus challenge. Nat Med 15: 293–299.

118. Hansen, SG, Ford, JC, Lewis, MS, Ventura, AB, Hughes, CM, Coyne-Johnson, L et al. (2011). Profound early control of highly pathogenic SIV by an effector memory T cell vaccine. Nature 473: 523–527.

122. Hansen, SG, Piatak, M Jr; Ventura, AB, Hughes, CM, Gilbride, RM, Ford, JC et al. (2013). Immune clearance of highly pathogenic SIV infection. Nature 502: 100–104.

126. Ondondo, BO (2014). The influence of delivery vectors on HIV vaccine efficacy. J Virol 88: 402–411.

127. Safrit, JT, Fast, PE, Gieber, L, Kuipers, H, Dean, HJ and Koff, WC (2016). Status of vaccine development for simian immunodeficiency virus. J Virol 86: 749–851.

131. Barouch, DH, Liu, J, Peter, L, Abbink, P, Iampietro, MJ, Cheung, A et al. (2011). Profound early control of highly pathogenic SIV by an effector memory T-cell vaccine. Nature 473: 523–527.

136. Kwa, S, Lai, L, Gangadhara, S, Siddiqui, M, Pillai, VB, Labranche, C et al. (2013). Induction of broadly targeted CD8+ T cell responses restricted by major histocompatibility complex class I. Proc Natl Acad Sci USA 110: 8580–8585.

137. Lai, L, Kwa, S, Kozlowski, PA, Montefiori, DC, Ferrari, G, Johnson, WE et al. (2013). Mucosal priming with adenovirus serotype 26 HIV-1 vaccination of humans. J Virol 87: 6620–6636.

138. Schell, JB, Rose, NF, Bahl, K, Diller, K, Buonocore, L, Hunter, M et al. (2013). First-human evaluation of the safety and immunogenicity of a recombinant adenovirus serotype 26 HIV-1 vaccine. J Virol 87: 6572–6580.

140. Richman, DD, Wrin, T, Little, SJ and Petropoulos, CJ (2003). Rapid evolution of the immune response to HIV type 1 infection. Proc Natl Acad Sci USA 100: 4144–4149.

143. Burns, DP and Desrosiers, RC (1991). Selection of genetic variants of simian immunodeficiency virus in persistently infected rhesus monkeys. J Virol 65: 1843–1854.

144. Burton, DR and Hansgamant, J (2016). Broadly neutralizing antibodies to HIV and their role in vaccine design. Annu Rev Immunol 34: 635–659.

145. Sanchez-Merino, V, Fabra-Garcia, A, Gonzalez, N, Nicolas, D, Merino-Mansilla, A, Manzano, C et al. (2016). Detection of broadly neutralizing activity within the first months of HIV-1 infection. J Virol 90: 5231–5245.

146. Doria-Rose, NA, Klein, RM, Manion, MM, O’Dell, S, Phogat, A, Chakrabarti, B et al. (2009). Frequency and phenotypic diversity of human immunodeficiency virus envelope-specific B cells from patients with broadly neutralizing antibodies. J Virol 83: 188–199.

147. Lai, L, Kwa, S, Kozlowski, PA, Montefiori, DC, Ferrari, G, Johnson, WE et al. (2013). Antibodies in HIV-1 vaccine development and therapy. Science 341: 1199–1204.

148. Kozlowski, PA, Montefiori, DC, Ferrari, G, Johnson, WE et al. (2013). Antibodies in HIV-1 vaccine development and therapy. Science 341: 1199–1204.

149. Klein, F, Dinkin, R, Scheid, JF, Gaebeler, C, Mouquet, H, Georgiev, IS et al. (2013). Somatic mutations of the immunoglobulin framework are generally required for broad and potent HIV-1 neutralization. Cell 153: 126–138.

150. Kuipers, H, Dean, HJ and Koff, WC (2016). Status of vaccine development for simian immunodeficiency virus. J Virol 86: 749–851.

151. Kwa, S, Lai, L, Gangadhara, S, Siddiqui, M, Pillai, VB, Labranche, C et al. (2013). Induction of broadly targeted CD8+ T cell responses restricted by major histocompatibility complex class I. Proc Natl Acad Sci USA 110: 8580–8585.

152. Lai, L, Kwa, S, Kozlowski, PA, Montefiori, DC, Ferrari, G, Johnson, WE et al. (2013). Prevention of infection by a granulocyte-macrophage colony-stimulating factor co-expressing DNA-modified vaccinia Ankara simian immunodeficiency virus vaccine. J Virol 87: 10780–10786.

153. Kepler, TB, Liao, HX, Alam, SM, Bhaskarabhatla, R, Zhang, R, Yandava, C et al. (2014). Immunoglobulin gene insertions and deletions in the affinity maturation of HIV-1 broadly reactive neutralizing antibodies. Cell Host Microbe 16: 304–313.

154. Kepler, TB, Liao, HX, Alam, SM, Bhaskarabhatla, R, Zhang, R, Yandava, C et al. (2014). Immunoglobulin gene insertions and deletions in the affinity maturation of HIV-1 broadly reactive neutralizing antibodies. Cell Host Microbe 16: 304–313.

155. Kwa, S, Lai, L, Gangadhara, S, Siddiqui, M, Pillai, VB, Labranche, C et al. (2014). CD40L-adjuvanted DNA-modified vaccinia virus Ankara simian immunodeficiency virus SIV239 vaccine enhances SIV-specific humoral and cellular immunity and improves protection against a heterologous SIV/WEE660 mucosal challenge. J Virol 88: 9579–9589.

156. Lafon, MC, Bolognesi, D, Luciano, G, Houlden, T, Van der Linden, P et al. (2015). The neutralization breadth of HIV-1 develops significantly during the first 6 months of HIV-1 infection. J Virol 89: 1843–1854.

157. Rappuoli, R, Bottomly, MJ, O’Dro, U, Finco, O and De Gregorio, E (2016). Reverse vaccinology 2.0: Human immunology instructs vaccine antigen design. Exp Med 213: 469–481.

158. MacLeod, DT, Choi, NM, Briney, B, Garces, F, Ver, LS, Landais, E et al.; IAV Protocol C Investigators & The IAV HIV Research Network. (2016). Early antibody lineage diversification and independent limb maturation lead to broad HIV-1 neutralization targeting the Env high-mannose patch. Immunity 44: 1215–1226.

159. Gorman, J, Soto, C, Yang, MM, Davenport, TM, Landais, E, Briney, B, Garces, F et al.; IAV Protocol C Investigators & The IAV HIV Research Network. (2016). Early antibody lineage diversification and independent limb maturation lead to broad HIV-1 neutralization targeting the Env high-mannose patch. Immunity 44: 1215–1226.

160. Jardine, JG, Ota, T, Sok, D, Pauthner, M, Kulp, DW, Kalyuzhniy, O et al. (2015). HIV-1 VACCINES. Priming a broadly neutralizing antibody response to HIV-1 using a germline-targeting immunogen. Science 349: 156–161.

161. Sanders, RW, van Gils, MJ, Derking, R, Sok, D, Ketas, T, Burghardt, D et al. (2015). HIV-1 VACCINES. HIV-1 neutralizing antibodies induced by native-like envelope trimers. Science 349: aaa4223.

162. Dosenovic, P, von Boehmer, L, Escola, A, Jardin, J, Freund, NT, Gitlin, AD et al. (2015). Immunization for HIV-1 broadly neutralizing antibodies in human Ig knockin mice. Cell 161: 1505–1515.

163. Doria-Rose, NA, Schramms, CA, Gorman, J, Moore, PL, Bhiman, JN, Dekosky, B et al.; NISC Comparative Sequencing Program. (2014). Developmental pathway for potent V1V2-directed HIV-neutralizing antibodies. Nature 509: 55–62.

164. Geogiou, G, Ippolito, GC, Beausang, J, Ushe, CE, Wardemann, H and Quake, SR (2014). The promise and challenge of high-throughput sequencing of the antibody repertoire. Nat Biotechnol 32: 158–160.

165. Jardine, JG, Ota, T, Sok, D, Pauthner, M, Kulp, DW, Kalyuzhniy, O et al. (2015). HIV-1 VACCINES. Priming a broadly neutralizing antibody response to HIV-1 using a germline-targeting immunogen. Science 349: 156–161.

166. Sanders, RW, van Gils, MJ, Derking, R, Sok, D, Ketas, T, Burghardt, D et al. (2015). HIV-1 VACCINES. HIV-1 neutralizing antibodies induced by native-like envelope trimers. Science 349: aaa4223.

167. Dosenovic, P, von Boehmer, L, Escola, A, Jardin, J, Freund, NT, Gitlin, AD et al. (2015). Immunization for HIV-1 broadly neutralizing antibodies in human Ig knockin mice. Cell 161: 1505–1515.
14

170. Wu, X., Zhou, T., Zhu, J., Zhang, B., Georgiev, I., Wang, C. et al.; NISC Comparative Sequencing Program. (2011). Focused evolution of HIV-1 neutralizing antibodies revealed by structures and deep sequencing. Science 333: 1593–1602.

171. Kwong, PD, Mascola, JR and Nabel, GJ (2013). Broadly neutralizing antibodies and the search for an HIV-1 vaccine: the end of the beginning. Nat Rev Immunol 13: 693–701.

172. Shcherbakov, DN, Bakulina, AY, Karpenko, LI and Ilyichev, AA (2015). Broadly neutralizing antibodies against HIV-1 as a novel aspect of the immune response. Acta Naturae 7: 11–21.

173. Burton, DR, Babäs, CF, 3rd, Persson, MA, Koenig, S, Channock, RM, and Lerner, RA (1991). A large array of human monoclonal antibodies to type 1 human immunodeficiency virus from combinatorial libraries of asymptomatic seropositive individuals. Proc Natl Acad Sci USA 88: 10134–10137.

174. Babäs, CF, 3rd, Bjorglin, E, Choi, F, Dunlop, N, Cababa, D, Jones, TM, et al. (1992). Recombinant human Fab fragments neutralize type 1 human immunodeficiency virus in vitro. Proc Natl Acad Sci USA 89: 9339–9343.

175. Muster, T, Steindl, F, Purtscher, M, Tikoła, A, Klima, A, Himmler, G et al. (2004). Efficient neutralization of primary isolates of HIV-1 by a recombinant human monoclonal antibody. Science 305: 359–361.

176. Burton, DR, Piyat, J, Kudori, R, Sharp, SJ, Thornton, GT, Parren, PW et al. (1994). Efficient neutralization of gp120 neutralizing epitope on gp41 of human immunodeficiency virus type 1. J Virol 68: 1024–1027.

177. Muster, T, Guinea, R, Tikoła, A, Purtscher, M, Klima, A, Steindl, F et al. (1994). Cross-neutralizing activity against divergent human immunodeficiency virus type 1 isolates induced by a gp41-specific human monoclonal antibody. J Virol 68: 1028–1034.

178. Conley, AJ, Kessler, JA, 2nd, Boots, LJ, Tung, JS, Arnold, BA, Keller, PM, et al. 1996. Neutralization of divergent human immunodeficiency virus type 1 variants and primary isolates by IA41-2F5, an anti-gp41 human monoclonal antibody. Proc Natl Acad Sci USA 91: 3348–3352.

179. Tikoła, A, Purtscher, M, Muster, T, Ballaun, C, Buchacher, A, Sullivan, N et al. (1996). Human monoclonal antibody 2G12 defines a distinctive neutralization epitope on the gp120 glycoprotein of human immunodeficiency virus type 1. J Virol 70: 1100–1108.

180. Wick, MB, Labrijn, AF, Wang, M, Speleman, C, Saphire, EO, Binley, JM et al. (2001). Broadly neutralizing antibodies targeted to the membrane-proximal external region of human immunodeficiency virus type 1 glycoprotein gp41. J Virol 75: 10892–10905.

181. Parren, PW, Gauduin, MC, Koup, RA, Poignard, P, Fiscarco, P, Burton, DR et al. (1997). Relevance of the antibody response against human immunodeficiency virus type 1 enevelope to vaccine design. Immunol Lett 57: 105–112.

182. Walker, LM, Phogat, SK, Chan-Hui, PY, Wagner, D, Phung, P, Goss, JL et al.; Protocol G Principal Investigators. (2009). Broad and potent neutralizing antibodies from an African donor reveal a new HIV-1 vaccine target. Science 326: 285–289.

183. Wu, X, Yang, ZY, Li, Y, Hogerkerp, CM, Schief, WR, Seaman, MS et al. (2010). Rational design of envelope identifies broadly neutralizing human monoclonal antibodies to HIV-1. Science 329: 856–861.

184. Scheid, JF, Mouquet, H, Ueberheide, B, Diskin, R, Klein, F, Oliveira, TY et al. (2011). Sequence and structural convergence of broad and potent HIV antibodies that mimic CD4 binding. Science 333: 1633–1637.

185. Klein, F, Gaebler, C, Mouquet, H, Sather, D, Lehmann, C, Scheid, JF et al. (2012). Broad neutralization by a combination of antibodies recognizing the CD4 binding site and a new conformational epitope on the HIV-1 envelope protein. J Exp Med 209: 1469–1479.

186. Walker, LM, Huber, M, Doores, KJ, Falkowska, E, Pejchal, R, Julien, JP et al.; Protocol G Principal Investigators. (2011). Broad neutralization coverage of HIV by multiple highly potent antibodies. Nature 477: 466–470.

187. Pejchal, R, Doores, KJ, Walker, LM, Khayat, R, Huang, PS, Wang, SK et al. (2011). A potent and broad neutralizing antibody recognizes and penetrates the HIV glycan shield. Science 334: 1057–1061.

188. Mouquet, H, Schraft, L, Eulner, Z, Liu, Y, Eden, C, Scheid, JF et al. (2012). Complex-type N-glycan recognition by potent broadly neutralizing HIV antibodies. Proc Natl Acad Sci USA 109: E626–E637.

189. Huang, J, O'fer, G, Laub, L, Merker, D, Karia-Rose, NA, Longo, NS et al. (2012). Broad and potent neutralization of HIV-1 by a gp41-specific human antibody. Nature 491: 406–412.

190. Kwon, YD, Georgiev, IS, O'fer, G, Zhang, B., Asokan, M, Bailey, RT et al. (2016). Optimization of the Solubility of HIV-1 Neutralizing Antibody 10E8 through Somatic Variation and Structure-Based Design. J Virol 90: 5899–5914.

191. Huang, J, Kang, BH, Pancera, M, Lee, JH, Tong, T, Feng, Y et al. (2014). Broad and potent HIV-1 neutralization by a human antibody that binds the gp41-gp120 interface. Nature 518: 138–142.

192. Sok, D, van Gils, MJ, Fauthner, M, Julien, JP, Saye-Francois, KL, Hsueh, J et al. (2014). Recombinant HIV envelope trimers selects for quaternary-dependent antibodies targeting the trimer apex. Proc Natl Acad Sci USA 111: 17624–17629.
217. Boumazos, S, DiLillo, DJ and Ravetch, JV (2015). The role of Fc-Fc interactions in IgG-mediated microbial neutralization. J Exp Med 212: 1361–1369.

218. Armbruster, C, Stiegler, GM, Vcelar, BA, Jäger, W, Michael, NL, Vetter, N et al. (2002). A phase I trial with two human monoclonal antibodies (aiMab 2F5, 2G12) against HIV-1. AIDS 16: 227–233.

219. Armbruster, C, Stiegler, GM, Vcelar, BA, Jäger, W, Köl ler, U, Jilch, R et al. (2004). Passive immunization with the anti-HIV-1 human monoclonal antibody (aiMab) 4E10 and the aiMab combination 4E10/2F5/2G12. Antimicrob Chemother 54: 915–920.

220. Stephenson, KE and Barouch, DH (2016). Broadly neutralizing antibodies for HIV eradication.Curr HIV/AIDS Res 13: 31–37.

221. Tkola, A, Kuster, H, Rupert, J, Jos, B, Fischer, M, Leemann, C et al. (2005). Delay of HIV-1 rebound after cessation of antiretroviral therapy through passive transfer of human neutralizing antibodies. Nat Med 11: 615–622.

222. Mehandru, S, Vcelar, B, Win, T, Stiegler, G, Joos, B, Mohr, H et al. (2007). Adjunctive passive immunotherapy in human immunodeficiency virus type 1-infected individuals treated with antiretroviral therapy during acute and early infection. J Virol 81: 11016–11031.

223. Lu, CL, Murakowski, DK, Bournazos, S, Schoofs, T, Sarkar, D, Halper-Stromberg, A et al. (2016). Engineered bispecific antibody Fc variants with enhanced effector function. MAbs 8: 157–173.

224. Balakrishnan, B and Jayandharan, GR (2014). Basic biology of adeno-associated virus (AAV) vectors used in gene therapy. Curr Gene Ther 14: 86–100.

225. Daya, S and Berms, KI (2008). Gene therapy using adeno-associated virus vectors. Microbiol Rev 72: 539–553.

226. Bournazos, S, Gazumyan, A, Seaman, MS, Nussenzweig, MC and Ravetch, JV (2016). Human IgG-mediated microbial neutralization. Proc Natl Acad Sci USA 103: 8875–8880.

227. Ackerman, ME, Dugast, AS and Alter, G (2012). Emerging concepts on the role of innate immunity in the prevention and control of HIV infection. Annu Rev Med 63: 113–130.

228. Schaefer, W, Regula, JT, Bahn er, M, Schanz er, J, Crossad e, R, Dunn, H et al. (2011). Immunoglobulin domain crossover as a generic approach for the production of bispecific IgG antibodies. Proc Natl Acad Sci USA 108: 11187–11192.

229. Moore, GL, Chen, H, Karki, S and Lazar, GA (2010). Engineered Fc variant antibodies with enhanced ability to recruit complement and mediate effector functions. MABs 2: 181–189.

230. Stroh, WR (2009). Optimization of Fc-mediated effector functions of monoclonal antibodies. Curr Opin Biotechnol 20: 685–691.

231. Kottermann, MA, Chalberg, TW and Schaffer, DV (2015). Viral vectors for gene therapy: translational and clinical outlook. Annu Rev Biomed Eng 17: 63–89.

232. Samulski, RJ and Muzycka, N (2014). AAV-mediated gene therapy for research and therapeutic purposes. Annu Rev Virol 1: 427–451.

233. Stiegler, G, Armbruster, C, Vcelar, B, Stoiber, H, Kunert, R, Michael, NL et al. (2016). Delay of HIV-1 rebound after cessation of antiretroviral therapy through passive transfer of human neutralizing antibodies. Nat Med 11: 615–622.

234. Ledgerwood, JE, Coates, EE, Yamshchikov, G, Saunders, JG, Holman, L, Enama, ME et al. (2016). Delay of HIV-1 infection in vitro by serum from HIV-1-infected and passively immunized nonhuman primates. Proc Natl Acad Sci USA 113: 157–162.

235. Wang, D, Zhong, L, Nahid, MA and Gao, G (2014). The potential of adeno-associated viral vectors for gene delivery to muscle tissue. Expert Opin Drug Deliv 11: 345–364.

236. Balakrishnan, B and Jayandharan, GR (2014). Basic biology of adeno-associated virus (AAV) vectors used in gene therapy. Curr Gene Ther 14: 86–100.

237. Mangozzi, F and High, KA (2011). Therapeutic in vivo gene transfer for genetic disease using AAV: progress and challenges. Nat Rev Genet 12: 341–355.

238. Balakrishnan, B and Jayandharan, GR (2014). Basic biology of adeno-associated virus (AAV) vectors used in gene therapy. Curr Gene Ther 14: 86–100.
270. MacLaren, RE, Groppe, M, Barnard, AR, Cottrill, CL, Tilmachov, T, Seymour, L et al. (2014). Retinal gene therapy in patients with choroideremia: initial findings from a phase 1/2A clinical trial. Lancet 383: 1129–1137.

271. Bowles, DE, McPhee, SW, Li, C, Gray, SJ, Samulski, JJ, Camp, AS et al. (2012). Phase 1 gene therapy for Duchenne muscular dystrophy using a translocation optimized AAV vector. Mol Ther 20: 443–455.

272. Maguire, AM, Simondon, F, Pierce, EA, Pugh, EN Jr, Mingozzi, F, Bennuci, J et al. (2008). Safety and efficacy of gene transfer for Leber’s congenital amaurosis. N Engl J Med 358: 2436–2447.

273. Jacobson, SG, Cideciyan, AV, Ratnakaram, R, Heon, E, Schwartz, SB, Roman, AJ et al. (2012). Gene therapy for leber congenital amaurosis caused by RP65 mutations: safety and efficacy in 15 children and adults followed up to 3 years. Arch Ophthalmol 130: 9–24.

274. LeWitt, PA, Rezai, AR, Leehey, MA, Ojemann, SG, Flaherty, AW, Eskandar, EN et al. (2012). Glybera finally recommended for approval as the first gene therapy drug in the European union. Transl Med 77: 309–319.

275. Bennett, J, Asharti, M, Wellman, J, Marshall, KA, Cyckowski, LL, Chung, DC et al. (2012). AAV2 gene therapy readministration in three adults with congenital blindness. Sci Transl Med 4: 120ra15.

276. Ylä-Herttuala, S (2012). Gene therapy for leber congenital amaurosis caused by RP65 mutations. Mol Ther 20: 443–455.

277. Saari, RA, Stewart, MW, Narvaez, MJ, Zabel, NG et al. (2011). Innate immune responses to AAV vectors. Front Immunol 2: 424–478.

278. Söderlund, SP, Fuchs and RC Desrosiers (2015). Promise and problems associated with the use of recombinant AAV for the delivery of anti-HIV antibodies. SP Fuchs and RC Desrosiers (2016) 16068 Official journal of the American Society of Gene & Cell Therapy.
Petry, H, Brooks, A, Orme, A, Wang, P, Liu, P, Xie, J et al. (2008). Effect of viral dose on neutralizing antibody response and transgene expression after AAV1 vector re-administration in mice. J Virol 72: 9795–9805.

Manning, WC, Zhou, S, Bland, MP, Escobedo, JA and Dwarki, V (1998). Transient immunosuppression allows transgene expression following readministration of adeno-associated viral vectors. Hum Gene Ther 9: 477–485.

Habert, CL, Standaert, TA, Wilson, CB and Miller, AD (1998). Successful readministration of adeno-associated virus vectors to the mouse lung requires transient immunosuppression during the initial exposure. J Virol 72: 1733–1742.

Chirmule, N, Xiao, W, Truneh, A, Schnell, MA, Hughes, JV, Zoltick, P et al. (1998). Mol Ther Methods Clin Dev 16068: 138–145.

Baker, MP, Reynolds, HM, Lumicisi, B and Bryson, C (2010). Immunogenicity of adeno-associated virus vectors 1 and 2. J Virol 84: 5385–5394.

Sok, D, Laserson, U, Laserson, J, Liu, Y, Vigneault, F, Julien, J et al. (2013). The effects of somatic hypermutation on neutralization and binding in the PGT121 family of broadly neutralizing HIV antibodies. PLoS Pathog 9: e1003754.

Francesco, M, Scheid, JF, Zoller, MJ, Krogsgaard, M, Ott, RG, Shukair, S et al. (2010). Polyeffectivity increases the apparent affinity of anti-HIV antibodies by heterologation. Nature 467: 591–595.

Martinez-Navio, JM, Fuchs, SP, Pedrero-López, S, Rakas, EG, Gao, G and Desrosiers, RC (2016). Host anti-antibody responses following adeno-associated virus-mediated delivery of antibodies against HIV and SIV in Rhesus monkeys. Mol Ther 24: 76–86.

Breden, F, Lepik, C, Longo, NS, Montero, M, Lipsky, PE and Scott, JK (2011). Comparison of antibody repertoire induced by HIV-1 infection, other chronic and acute infections, and systemic autoimmune disease. PLoS One 6: e16857.

Harding, FA, Stickler, MM, Rayo, J and DuBridge, RB (2010). The immunogenicity of humanized and fully human antibodies: residual immunogenicity resides in the CDR regions. Mabs 2: 256–265.

Krishna, M and Nadler, SG (2016). Immunogenicity to biotherapeutics - the role of anti-drug immunogenicity complexes. Front Immunol 7: 21.

Scott, DW and De Groot, S (2010). Can we prevent immunogenicity of human protein drugs? Ann Rheum Dis 69 Suppl 1: i72–i76.

van Beers, MM and Bardor, M (2012). Minimizing immunogenicity of biopharmaceuticals by controlling critical quality attributes of proteins. Biotechnol J 7: 1473–1484.

Baker, MP, Reynolds, HM, Lumicisi, B and Bryson, C (2010). Immunogenicity of protein therapeutics: the key causes, consequences and challenges. Self Nonself 1: 314–322.

Bartelds, GM, Wolbink, GJ, Stapel, S, Aarden, L, Lems, WF, Dijkmans, BA et al. (2006). High levels of human anti-human antibodies to adalimumab in a patient not responding to adalimumab treatment. Ann Rheum Dis 65: 1294–1295.

Wang, W, Wang, EQ and Balthasar, JP (2008). Monoclonal antibody pharmacokinetics and related pharmacodynamic considerations. Clin Pharmacol Ther 84: 548–558.

Radstake, TR, Svensson, M, Eijjsbouts, AM, van den Hoogen, FH, Ennevel, C, van Riel, PL et al. (2009). Formation of antibodies against infliximab and adalimumab strongly correlates with functional drug levels and clinical responses in rheumatoid arthritis. Ann Rheum Dis 68: 1739–1745.

van Meer, PJ, Kooijman, M, Brinks, V, Gispens-de Vied, CC, Silva-Lima, B, Boors, EH et al. (2013). Immunogenicity of mAbs in non-human primates during nonclinical safety assessment. Mabs 5: 810–816.

Hwang, WY and Foote, J (2005). Immunogenicity of engineered antibodies. Methods 36: 3–10.

Thullier, P, Chahboun, S and Pelat, T (2010). A comparison of human and macaque (Macaca mulatta) immunoglobulin germline V regions and their implications for antibody engineering. Mabs 2: 528–538.

West, AP, Gilmel, RP, Ginapraparam, PN and Bjorkman, PJ (2012). Single-chain Fv-based anti-HIV proteins: potential and limitations. J Virol 86: 195–202.

Rudicell, RS, Kwon, YD, Ko, SY, Pegu, A, Louder, MK, Georgiev, IS et al. (2009). Function and dynamics of human anti-human antibodies to adalimumab, etanercept and infliximab: residual immunogenicity resides in the CDR regions. J Immunol 182: 7752–7759.

Takemoto, R, Kato, S, Muroya, T, Nagase, T, Gotoda, T et al. (2010). Lack of immunogenicity after intravenous (IV) delivery of rAAV to nonhuman primate skeletal muscle. Mol Ther 18: 151–160.

Kim, KJ, Mizzakui, U, Usabe, M, Toda, Y, Shimoda, K, Yoshida, A et al. (2006). Induction of robust immune responses against human immunodeficiency virus is supported by the inherent tropism of adeno-associated virus type 5 for dendritic cells. J Virol 80: 11899–11910.

Veron, P, Allo, V, Rivière, C, Bernard, J, Douar, AM and Masurier, C (2007). Major subsets of human dendritic cells are efficiently transduced by self-complementary adeno-associated virus vectors. Mol Ther 15: 338–358.

Chen, Q, Li, Z, Zhang, Y, Li, Z, Wang, W, Xiong, Y, Xu, Y et al. (2007). Effective gene therapy for haemophilic mice with pathogenic factor IX antibodies. EMBO Mol Med 5: 1698–1709.

Maxeiner, S, Vanderberg, LH, Xiao, R, Bell, P, Nam, HJ, Agbandje-McKenna, M et al. (2009). Adeno-associated virus capsid structure drives CD4-dependent CD8+ T cell response to vector-encoded proteins. J Immunol 182: 6051–6060.

Mays, LE and Wilson, JM (2011). The complex and evolving story of T cell activation to AAV vector-encoded transgene products. Mol Ther 19: 16–27.

Lu, Y and Song, S (2009). Distinct immune responses to transgene products from AAV1 and AAV8 vectors. Proc Natl Acad Sci USA 106: 17158–17162.

Sen, D, Gadkari, RA, Sudha, G, Gabriel, N, Kumar, YS, Selot, R et al. (2013). Targeted modifications in adeno-associated virus serotype 8 capsids improves its hepatic gene transfer efficiency in vivo. Hum Gene Ther Methods 24: 104–116.

Wang, L, Louboutin, JP, Bell, P, Greig, JA, Li, Y, Wu, D et al. (2011). Muscle-directed gene therapy for hemophilia B with more efficient and less immunogenic AAV vectors. J Thromb Haemost 9: 26–30.

Wu, T, Töpper, K, Lim, SW, Li, H, Bhan, A, Zhou, YY et al. (2012). Self-complementary AAVs induce more potent transgene product-specific immune responses compared to a single-stranded genome. Mol Ther 20: 572–579.

Greig, JA, Peng, H, Ohlstein, J, Medina-Jaszek, CA, Ahooka, O, Mentzinger, A et al. (2014). Intramuscular injection of AAV8 in mice and macaques is associated with substantial hepatic targeting and transgene expression. PLoS One 9: e112266.

Li, C, Dippromo, N, Bowles, DE, Hirsch, ML, Monahan, PE, Asokan, A et al. (2012). Single amino acid modification of adeno-associated virus capsid changes transcription and hepatic immune profiles. J Virol 86: 7752–7759.

Toromanoff, A, Adjali, O, Archer, T, Hill, M, Guigand, L, Chenuaud, P et al. (2010). Lack of immunotolerance after intravenous (IV) delivery of rAAV to nonhuman primate skeletal muscle. Mol Ther 18: 151–160.

Kim, K, Mizzakui, U, Usabe, M, Toda, Y, Shimoda, K, Yoshida, A et al. (2006). Induction of robust immune responses against human immunodeficiency virus is supported by the inherent tropism of adeno-associated virus type 5 for dendritic cells. J Virol 80: 11899–11910.
Promise and problems associated with the use of recombinant AAV for the delivery of anti-HIV antibodies

SP Fuchs and RC Desrosiers

377. Qiao, C, Zhang, W, Yuan, Z, Shin, JH, Li, J, Jayandharan, GR et al. (2010). Adeno-associated virus serotype 6 capsid tyrosine-to-phenylalanine mutations improve gene transfer to skeletal muscle. Hum Gene Ther 21: 1343–1348.

378. Vercauteren, K, Hoffman, BE, Zolotukhin, I, Keeler, GD, Xiao, JW, Basner-Tschakarjan, E et al. (2016). Superior in vivo transduction of human hepatocytes using engineered AAV3 capsid. Mol Ther 24: 1042–1049.

379. Majowicz, A, Maczuga, P, Kwikkers, KL, van der Marel, S, van Logtenstein, R, Petry, H et al. (2013). Mir-142-3p target sequences reduce transgene-directed immunogenicity following intramuscular adeno-associated virus 1 vector-mediated gene delivery. J Gene Med 15: 219–232.

380. Boisgerault, F, Gross, DA, Ferrand, M, Poupiot, J, Darocha, S, Richard, I et al. (2013). Prolonged gene expression in muscle is achieved without active immune tolerance using microRNA 142.3p-regulated rAAV gene transfer. Hum Gene Ther 24: 393–405.

381. Wang, X, Terhorst, C and Herzog, RW (2016). In vivo induction of regulatory T cells for immune tolerance in hemophilia. Cell Immunol 301: 18–29.

382. Sharland, A, Logan, GJ, Bishop, A and Alexander, IE (2010). Liver-directed gene expression using recombinant AAV 2/8 vectors—a tolerogenic strategy for gene delivery? Discov Med 9: 519–527.

383. Sack, BK, Herzog, RW, Terhorst, C and Markusic, DM (2014). Development of gene transfer for induction of antigen-specific tolerance. Mol Ther Methods Clin Dev 1: 14013.

384. Mueller, C, Chulay, JD, Trapnell, BC, Humphries, M, Carey, B, Sandhaus, RA et al. (2013). Human Treg responses allow sustained recombinant adeno-associated virus-mediated transgene expression. J Clin Invest 123: 5310–5318.

385. Mays, LE, Wang, L, Lin, J, Bell, P, Crawford, A, Wherry, EJ et al. (2014). AAV8 induces tolerance in murine muscle as a result of poor APC transduction, T cell exhaustion, and minimal MHCI upregulation on target cells. Mol Ther 22: 28–41.

386. LoDuca, PA, Hoffman, BE and Herzog, RW (2009). Hepatic gene transfer as a means of tolerance induction to transgene products. Curr Gene Ther 9: 104–114.

387. Büning, H and Schmidt, M (2015). Adeno-associated vector toxicity—to be or not to be? Mol Ther 23: 1673–1675.