Published in final edited form as:

Curr Opin Immunol. 2015 August ; 35: 113–122. doi:10.1016/j.coi.2015.06.014.

Engineering Humoral Immunity as Prophylaxis or Therapy

Cailin E. Deal¹ and Alejandro B. Balazs^{1,*}

¹Ragon Institute of MGH, MIT & Harvard, 400 Technology Sq., Cambridge, MA 02139

Abstract

Purpose of the review—In this review, we will discuss the field of engineered humoral immunity with an emphasis on recent work using viral vectors to produce antibodies *in vivo*. As an alternative to passive transfer of monoclonal antibody protein, a transgene encoding an antibody is delivered to cells via vector transduction, resulting in expression and secretion by the host cell. This review will summarize the evidence in support of this strategy as an alternative to traditional vaccines against infection and as novel therapeutics for a variety of diseases.

Recent findings—Historically, humoral immunity has been engineered through vaccination and passive transfer of monoclonal antibodies. However, recent work suggests that vectors can be used to deliver transgenes encoding broadly neutralizing antibodies to nonhematopoietic tissues and can mediate long-term expression that is capable of preventing or treating infectious diseases. The production of engineered monoclonal antibodies allows for precise targeting and elimination of aberrant self-proteins that are characteristic of certain neurodegenerative disease. This approach has also been successfully used to combat cancer and addiction in several animal models. Despite the wide array of expression platforms that have been described, adeno-associated virus vectors have emerged as the frontrunner for rapid clinical translation.

Summary—Recent advances in vector-mediated antibody expression have demonstrated the potential for such interventions to prevent infection and treat disease. As such, it offers an alternative to immunogen-based vaccine design and a novel therapeutic intervention by enabling precise manipulation of humoral immunity. Success translating these approaches to patients may enable the development of effective prevention against previously intractable pathogens that evade immunity such as HIV, influenza, malaria or HCV and may also enable new treatment options for neurodegenerative diseases such as Alzheimer's disease.

Keywords

Antibody gene transfer; vectored immunoprophylaxis; engineered humoral immunity; broadly neutralizing antibodies

*Direct all correspondence to: Ragon Institute of MGH, MIT & Harvard, 400 Technology Sq. Room 992, Cambridge, MA 02139; phone: (857) 268-7100; fax: (857) 268-7142; abalazs@mgh.harvard.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Conflicts of Interest
None.

Introduction

The humoral immune system is one of the first obstacles encountered by invading pathogens, thus playing a crucial role in preventing infection and maintaining human health. Immunological memory, particularly in the form of pre-existing antibodies, has been shown to form the basis of protection for nearly all vaccines in use today [1]. This ability to generate memory against previously encountered pathogens enabled the first practitioners in Asia to engineer immunity against smallpox in the fifteenth century through a process termed variolation [1]. By intentionally exposing patients to the relatively mild variola minor, they were able to induce protective immunity that prevented life-threatening smallpox infection by variola major [1]. Similarly, modern-day vaccines engineer immunity by exposing a patient to inactivated or attenuated whole pathogens, or recombinant components of pathogens that are known to elicit protective immunity. As a result, effective vaccines have been developed against many of the diseases for which natural infection results in immunity against re-infection. This practice has resulted in the global eradication of smallpox [2] and decreased incidence of diphtheria, measles, mumps, pertussis, poliomyelitis, rubella and tetanus [3]. However a number of diseases for which prior exposure is ineffective at preventing subsequent disease have proven more difficult targets. These include intractable pathogens that evade immunity, such as human immunodeficiency virus (HIV), malaria, hepatitis C virus (HCV), and influenza A virus (IAV) as well as complex diseases of self, such as cancer and neurodegenerative disorders.

Engineered Humoral Immunity Through Passive Transfer

Ideally, vaccination elicits a protective cellular and humoral response, however the protection raised by most of the currently licensed vaccines is largely antibody-dependent [1,4]. Importantly, neutralizing antibodies (nAb) alone have been shown to prevent the spread of many diseases within populations [4,5]. This antibody-based protection underlies passive transfer, whereby the administration of sera or purified antibodies into naïve patients transiently confers immunity. Technological advances including the development of hybridoma technology [6] and the “humanization” of antibodies has spawned a new class of antibody-based drugs which have demonstrated remarkable success in the clinic for a wide range of diseases.

While mAbs have several advantages over conventional small-molecule drugs, there are considerable disadvantages as well. Currently licensed mAbs have a typical half-life ranging from two days to one month *in vivo* [7], necessitating frequent dosing. However, specific mutations in the antibody Fc region have been described that prolong half-life up to five-fold by increasing the affinity of the antibody for the neonatal Fc receptor (FcRn) [8]. Despite the potential for less frequent dosing, high concentrations of most antibodies are necessary to achieve clinical efficacy, resulting in high materials costs [5,9]. Additionally, antibody use is further complicated by the requirement of cold-chain storage and trained medical personnel for administration [7], making such therapy difficult to implement in low-resource areas. For mAb therapy to become a widely administered intervention on a global scale, improved delivery approaches will be required.

Vectored Antibody Gene Delivery for Infectious Diseases

One alternative to passive transfer utilizes a vector for the delivery of transgenes encoding previously characterized antibodies. These transgenes direct the production of mAbs in non-hematopoietic cells, which secrete mAbs into the circulation or the local environment. A wide variety of vectors, each with distinct expression profiles, have been considered for this approach (Table 1).

Naked plasmid DNA offers simplicity of use, lack of immunogenicity and ease of large-scale production. Improvements in electroporation techniques has allowed for enhanced transfection of specific tissues *in vivo*. Electroporation of various monoclonal antibody transgenes into muscle has yielded production of mAb light and heavy chains (ie. Fab fragments), peaking at 50-200ng/mL in mice and 30-50ng/mL in sheep [10]. Optimization of the expression plasmid and electroporation conditions yielded 2-3µg/mL of the VRC01 HIV broadly neutralizing antibody (bNAb) in the plasma of mice 12 days post administration [11], demonstrating that electroporation of plasmid DNA encoding antibody can be used to rapidly produce mAb *in vivo*. However, the modest concentrations and transient nature of expression obtained with existing protocols limit the clinical potential of this technique.

Viruses have been exploited as vectors for many years owing to their highly evolved mechanisms for efficient delivery of genetic material to host cells. Lentiviral vectors, consisting of an extensively modified HIV-1-derived genome pseudotyped with vesicular stomatitis virus (VSV) G protein, represent an attractive means of integrating transgenes into the host genome, enabling long term gene expression in a wide variety of both dividing and nondividing cells. Such vectors have been used to transduce primary hematopoietic stem cells (HSC) with a transgene encoding the HIV bNAb b12, allowing for their differentiation into plasmablasts that secreted b12 *in vitro* [12]. Using a similar approach, B cells were engineered to secrete HIV bNAb 2G12 in a humanized mouse model, achieving concentrations of approximately 40ng/mL in plasma, which was sufficient to inhibit HIV infection *in vivo* [13]. However, all of these studies utilized *ex vivo* transduction, making widespread implementation of this approach challenging.

While lentivirus is well suited for long-term expression of mAb, adenoviral vectors have been shown to exhibit transient, but rapid gene expression ideal for responding to infectious disease outbreaks. Adenovirus serotype 5 (Ad5) encoding Palivizumab, a respiratory syncytial virus (RSV) mAb, produced detectable antibody expression as early as four days post-transduction and resulted in a 5.4 fold decrease in RSV titers in the lung four days post-challenge as compared to controls [14]. Ad5 has also been used to express a single-domain antibody specific for H5N1 influenza A virus (IAV) hemagglutinin (HA), which protected mice when administered 14 days prior to, or even 48 hours after, infection [15]. In another study, a mAb targeting the protective antigen of *Bacillus anthracis* was delivered by Ad5 that protected mice from toxin challenge between 1 day and 8 weeks post-administration, but which was no longer protective at 6 months [16].

Adeno-associated virus (AAV) has never been associated with any disease in humans and recombinant vectors derived from AAV (rAAV) result in stable gene expression in the absence of integration through formation of extrachromosomal concatamers of the delivered transgene sequences [17]. The serotype used to package the vector strongly influences its ability to transduce different tissues [17,18] and has been shown to play a significant role in the immunogenicity of the vector in various animal models [19,20]. AAV1 delivering lipoprotein lipase (LPL) was recently approved in Europe as the first ever gene therapy product for humans [21] and recent clinical trials testing AAV8 for the delivery of Factor IX for hemophilia has met with considerable success [22]. Given their clinical efficacy and favorable expression profile, AAV vectors have been extensively characterized as a platform for the delivery of mAbs *in vivo*. However, the packaging capacity of AAV is limited to 5 kilobases, presenting a significant obstacle to the efficient expression of both heavy and light chain. As a result, some groups have turned to smaller bivalent single chain antibodies (scFv) or immunoadhesins, chimeric antibody-like molecules combining the functional domain with the immunoglobulin constant domain [23-26]. However, others have employed heterologous viral sequences such as foot and mouth disease virus-derived 2A self-processing sequence (F2A) to express full-length antibodies from a single open reading frame [27], yielding greater than 1000 µg/mL of sustained mAb serum levels *in vivo* [27,28].

The earliest forms of AAV mediated gene antibody transfer were implemented as a dual-promoter vector whereby the heavy and light chain genes were transcribed independently. This yielded up to 8µg/mL of biologically active HIV bNAb for over 6 months in immunodeficient Rag mice [29]. In rhesus macaques, expression of SIV gp120-specific immunoadhesins peaked 3-4 weeks post transduction at 200µg/mL and was sustained at 20µg/mL for at least 4 years [30]. Out of nine monkeys challenged with SIV one month after AAV administration, six were completely protected from challenge. The three immunized macaques that became infected were later found to have developed an immune response against the immunoadhesin one week prior to challenge, suggesting that an anti-immunoadhesin response led to the observed failure of protection [30]. AAV vectors were also employed in a similar approach coined 'Vectored ImmunoProphylaxis' (VIP), whereby full-length human IgG bNAbs against HIV were expressed from an optimized transgene that utilized the F2A sequence to allow for the expression of independent heavy and light chains under a muscle-optimized promoter [31]. This transgene was packaged with an AAV8 capsid [19], leading to the production of mAb at serum concentrations greater than 100µg/mL for at least 52 weeks [31]. Using this system, two different humanized mouse models were protected from either IV challenge with a laboratory strain of HIV [31] or repetitive low-dose vaginal challenge with a more clinically relevant transmitted founder strain (REJO.c) [32]. Decreasing doses of AAV vector led to dose-dependent antibody expression, enabling a determination of the minimum protective dose *in vivo* for a number of antibodies [31,32]. AAV-vectored bNAbs have also been shown to work in conjunction with passive mAb transfer and HAART to maintain suppression of HIV replication in humanized mice [33]. Most recently, a synthetic fusion of CD4-Ig with a small CCR5-mimetic sulfopeptide (eCD4-Ig) was delivered by AAV and protected rhesus macaques from several infectious SHIV challenges suggesting that AAV-vectored synthetic proteins may be able to create effective HIV prophylaxis [34].

In addition to HIV, successful VIP has also been demonstrated against a number of other infectious diseases. Recently, sterilizing immunity was generated against a murine model of *Plasmodium falciparum* infection, the malaria parasite responsible for the highest mortality in humans [35], demonstrating the first known example where a parasitic disease was prevented by antibodies alone. Likewise, VIP-mediated expression of bNAbs against HCV conferred protection against viral challenge in humanized mice [36] and was able to abrogate ongoing HCV infection both *in vitro* and *in vivo*. Additionally, sera taken from mice given VIP expressing different bNAbs targeting IAV hemagglutinin (HA), were able to neutralize all H1, H2 and H5 strains tested, and antibody expression lasted well over a year post-AAV injection [37]. Interestingly, protection against influenza by VIP did not appear to inhibit the elicitation of novel immune responses, suggesting that VIP may be capable of augmenting immunity without abrogating endogenous immune responses [37]. Notably, this method also protected immunodeficient and older mice from disease, representing two particularly vulnerable human patient populations who are inadequately protected by traditional vaccination [37]. While intramuscular (IM) injection of AAV for expression of antibodies has been most common, other routes of administration and target sites have also been used successfully. One study used AAV9 to deliver IAV bNAb F16 by intranasal administration, which resulted in protection at the primary site of challenge [38]. AAV has also been used to generate long-term expression of an RSV antibody [14]. One study tested a combination of AAV and Ad5 to generate rapid protection against anthrax that lasted at least 26 weeks [16].

Vectored Antibody Delivery for Neurodegenerative Diseases

In addition to engineering immunity against infectious disease, the use of AAV as a means of creating desirable antibody specificities *in vivo* enables targeting of “self” proteins that would be difficult or impossible to target safely through traditional vaccination. As a result, there has been growing interest in utilizing this approach for the treatment and prevention of neurodegenerative diseases, which represent an increasing share of the healthcare burden in developed nations. For many such diseases, aggregation of misfolded proteins has been suggested as the underlying mechanism, making them ideal targets for mAbs that recognize the misfolded variants and prevent the formation of these aggregates. Small scFv antibody fragments lacking the Fc region, including intrabodies (iAb) that target antigens intracellularly, can distinguish highly homologous proteins, different conformations of the same protein and, in the case of some iAb, target proteins to distinct cellular compartments [39-41]. The genes encoding these scFv have been delivered by AAV vectors, representing a powerful new tool for treating or preventing neurodegenerative disease [42,43].

Huntington’s disease (HD) is caused by a mutation in the huntingtin protein (HTT) and is a model for numerous neurodegenerative disorders due to its simple autosomal dominant inheritance. Several anti-HTT iAbs have been generated [44] and two were tested in transgenic HD models using intrastriatal AAV2 delivery [45]. Of the iAbs tested, Happ1 ameliorated neuropathology in cell lines and conferred significant beneficial effects in a variety of motor and cognitive assays, significantly prolonging life span by 10 weeks in a mouse model of disease [45]. Another group delivering iAb C4 via intrastriatal AAV1

delivery demonstrated that early treatment of an HD mouse model, prevented cells from exhibiting nuclear aggregates and delayed aggregate accumulation [46].

Alzheimer's disease (AD) is a disorder characterized by the diffuse loss of neurons and accumulation of amyloid beta proteins (A β) in the brain. Recently, scFvs have been developed that target A β , which could reduce A β burden and possibly alleviate symptoms [47]. A transgenic mouse model for AD, given an intrahippocampal infusion of AAV1 encoding an A β -scFv exhibited lower levels of insoluble A β , increased numbers of microglia and demonstrated improved cognitive function [48]. While most studies have administered AAV vectors directly into the brain, this may pose a safety risk as intraventricular delivery of AAV5 led to an increase in hemorrhaging [23]. As an alternate route, IM injection of AAV2 expressing an A β -scFv was found to be as effective as intracranial administration in reducing physiologic and behavioral effects of AD without producing detectable inflammatory responses or microhemorrhages in the brain [24,49]. In a separate study, IM injection of AAV1 expressing a full-length A β -mAb resulted in sustained anti-A β levels above 100 μ g/mL in serum that were maintained for up to 64 weeks post-injection [50]. These levels were found to be effective in decreasing A β levels in the brain both prophylactically and therapeutically [50].

Emerging evidence suggests that misfolding of superoxide dismutase 1 (SOD1) is a common pathogenic event in amyotrophic lateral sclerosis (ALS)[51]. AAV encoding an scFv specific for misfolded SOD1, was injected intrathecally into an ALS mouse model, resulting in reduced neuronal stress, reduced levels of misfolded SOD1 in the spinal cord and attenuation of motor neuron loss (Table 1) [25]. Overall, this led to delayed disease onset and increased life span that directly correlated with antibody titer.

Similarly, this approach has also been investigated to combat prion disease, which is a neurodegenerative disorder caused by a conversion of cellular prion protein (PrP^c) into the misfolded, insoluble, PrP^{sc}. Mice inoculated peripherally with infectious prions were given an AAV vector expressing scFv targeting PrP^{sc}. These mice exhibited decreased PrP^{sc} burden and delayed onset of prion pathogenesis as determined by improvements of clinical signs (Table 1) [26,52].

Vectored Antibody Delivery for Cancer

As a result of improved understanding the molecular basis of cancer, antibody-based drugs have become the standard of care for many types of tumors. Human epidermal growth factor receptor type 2 (HER2) overexpression is associated with reduced survival in cases of human breast cancer. Clinical trials of trastuzumab, an mAb that targets an extracellular region of HER2, have been successful at steady state serum concentrations of greater than 10 μ g/mL [53]. The murine precursor to trastuzumab was encoded in an AAV rh.10 vector [54] and administered to C57BL/6 mice, resulting in serum concentrations near 35 μ g/mL within twelve weeks that were sustained for at least 56 weeks post injection. A single injection of this vector increased the survival of Balb/c nu/nu mice injected subcutaneously with Calu-3 tumor cells over-expressing HER2, demonstrating its efficacy at inhibiting cancer *in vivo* [54].

Similarly, cutaneous T-cell lymphoma (CTCL), exhibits over-expression of chemokine receptor 4 (CCR4), whose expression is limited amongst non-malignant cells [55]. AAV8 was used to express a humanized anti-CCR4 mAb in a tumor mouse model, resulting in reduced CCR4⁺ tumor growth and increased survival. A single IV injection resulted in 96µg/mL of mAb in serum, which was able to significantly reduce tumor growth and increase life-span as compared to control animals [56]. While these results have been promising, clinical translation of vectored antibody delivery for cancer will require the use of transient vectors or platforms for regulated mAb delivery.

Vectored Antibody Delivery for Addiction

Substance abuse creates a substantial healthcare burden and is the target of numerous pharmacological and behavioral interventions. Antibodies that target the addictive substance and prevent receptor signaling could offer a potential treatment, however repeated administration of mAb protein is impractical. Previous studies showed that AAVrh.10 expressing a high affinity anti-nicotine mAb, resulted in a serum concentration of 1.3 mg/mL that lasted for at least 18 weeks [57]. AAV-NIC9D9 mice had a majority of serum nicotine bound to the Fab within one minute after challenge with nicotine, resulting in brain concentrations of nicotine that were only 15% of what was observed in naïve controls. Importantly, the expression of this antibody also blocked nicotine-mediated alterations in arterial blood pressure, heart rate and locomotor activity, demonstrating its ability to obviate the physiological effects of nicotine [57]. Using a similar approach against cocaine, AAVrh.10 was engineered to express the high affinity anti-cocaine mAb GNC92H2 [58], leading to the expression of anti-cocaine antibodies for at least 24 weeks after IV administration and resulting in a 31-fold reduction in the ratio of brain to blood cocaine levels and reduced hyperactivity in treated mice as compared to controls [58].

Conclusion

Successful translation of vectored antibody gene delivery to patients is poised to redefine the landscape of immunological interventions by enabling precise engineering of the specificity and intensity of a desirable humoral response. It goes well beyond the ability of traditional vaccines to enable production of non-natural antibody architectures capable of discriminating between normal and aberrant forms of self-proteins. By circumventing the natural immune system, vectored antibody delivery has the potential to yield protection regardless of immune-status or age, allowing it to reach currently vulnerable populations of patients who cannot respond to vaccines and offering a possible alternative to existing therapies delivered by passive transfer.

Acknowledgements

Financial Support

This work was supported by the National Institute of Allergy and Infectious Disease (NIAID) Career Transition Award K22AI102769 and the National Institutes for Drug Abuse (NIDA) Avenir New Innovator Award DP2DA040254 as well as funding from the William F. Milton Fund, and the Charles H. Hood Foundation.

Abbreviations

rAAV	recombinant aden-associated virus
scAAV	selfcomplementary adeno-associated virus
Ad5	adenovirus serotype 5, fragmentantigen binding
scFv	single-chain variable fragment
CMV	cytomegalovirus
hPGK	human phosphoglycerate kinase promoter
EF1α	elongation factor 1 α
N/A	not applicable
MOI	multiplicity of infection
GC	genome copy
PFU	plaque forming unit
IM	intramuscular
IV	intravenous

References

- [1]. Amanna IJ, Slifka MK. Contributions of humoral and cellular immunity to vaccine-induced protection in humans. *Virology*. 2011; 411:206–215. [PubMed: 21216425]
- [2]. Fenner, F.; Henderson, DA.; Arita, I.; Jezek, Z.; Ladnyi, ID. Smallpox and its eradication/F. Fenner...[et al.]. 1988.
- [3]. Roush SW, Murphy TV. Vaccine-Preventable Disease Table Working Group, Historical comparisons of morbidity and mortality for vaccine-preventable diseases in the United States. *Jama*. 2007; 298:2155–2163. [PubMed: 18000199]
- [4]. Plotkin SA. Correlates of protection induced by vaccination. *Clin Vaccine Immunol*. 2010; 17:1055–1065. [PubMed: 20463105]
- [5]. Chames P, Van Regenmortel M, Weiss E, Baty D. Therapeutic antibodies: successes, limitations and hopes for the future. *Br. J. Pharmacol*. 2009; 157:220–233. [PubMed: 19459844]
- [6]. Köhler G, Milstein C. Continuous cultures of fused cells secreting antibody of predefined specificity. *Nature*. 1975; 256:495–497. [PubMed: 1172191]
- [7]. Keizer RJ, Huitema AD, Schellens JH, Beijnen JH. Clinical pharmacokinetics of therapeutic monoclonal antibodies. *Clinical Pharmacokinetics*. 2010; 49:493–507. [PubMed: 20608753]
- [8]. Zalevsky J, Chamberlain AK, Horton HM, Karki S, Leung IWL, Sproule TJ, et al. Enhanced antibody half-life improves in vivo activity. *Nature Biotechnology*. 2010; 28:157–159.
- [9]. Pescovitz MD. Rituximab, an anti-cd20 monoclonal antibody: history and mechanism of action. *Am. J. Transplant*. 2006; 6:859–866. [PubMed: 16611321]
- [10]. Tjelle TE, Corthay A, Lunde E, Sandlie I, Michaelsen TE, Mathiesen I, et al. Monoclonal antibodies produced by muscle after plasmid injection and electroporation. *Mol Ther*. 2004; 9:328–336. [PubMed: 15006599]
- [11]. Muthumani K, Flingai S, Wise M, Tingey C, Ugen KE, Weiner DB. Optimized and enhanced DNA plasmid vector based in vivo construction of a neutralizing anti-HIV-1 envelope glycoprotein Fab. *Hum Vaccin Immunother*. 2013; 9:2253–2262. [PubMed: 24045230] *This study is one of the first to describe an enhanced muscle electroporation strategy that rapidly generates Fab fragments *in vivo* for HIV prevention.

- [12]. Luo XM, Maarschalk E, O'Connell RM, Wang P, Yang L, Baltimore D. Engineering human hematopoietic stem/progenitor cells to produce a broadly neutralizing anti-HIV antibody after in vitro maturation to human B lymphocytes. *Blood*. 2009; 113:1422–1431. [PubMed: 19059876]
- [13]. Joseph A, Zheng JH, Chen K, Dutta M, Chen C, Stiegler G, et al. Inhibition of in vivo HIV infection in humanized mice by gene therapy of human hematopoietic stem cells with a lentiviral vector encoding a broadly neutralizing anti-HIV antibody. *Journal of Virology*. 2010; 84:6645–6653. [PubMed: 20410262]
- [14]. Skaricic D, Traube C, De B, Joh J, Boyer J, Crystal RG, et al. Genetic delivery of an anti-RSV antibody to protect against pulmonary infection with RSV. *Virology*. 2008; 378:79–85. [PubMed: 18556039]
- [15]. Tutykhina IL, Sedova ES, Gribova IY, Ivanova TI, Vasilev LA, Rutovskaya MV, et al. Passive immunization with a recombinant adenovirus expressing an HA (H5)-specific single-domain antibody protects mice from lethal influenza infection. *Antiviral Research*. 2013; 97:318–328. [PubMed: 23274786] *This paper demonstrated that Ad5-mediated antibody expression was able to function as a prevention and treatment for pandemic influenza.
- [16]. De BP, Hackett NR, Crystal RG, Boyer JL. Rapid/sustained anti-anthrax passive immunity mediated by co-administration of Ad/AAV. *Mol Ther*. 2008; 16:203–209. [PubMed: 18059375]
- [17]. WU Z, Asokan A, SAMULSKI R. Adeno-associated Virus Serotypes: Vector Toolkit for Human Gene Therapy. *Molecular Therapy*. 2006; 14:316–327. [PubMed: 16824801]
- [18]. Chao H, Liu Y, Rabinowitz J, Li C, Samulski RJ, Walsh CE. Several log increase in therapeutic transgene delivery by distinct adeno-associated viral serotype vectors. *Mol Ther*. 2000; 2:619–623. [PubMed: 11124063]
- [19]. Gao G-P, Alvira MR, Wang L, Calcedo R, Johnston J, Wilson JM. Novel adeno-associated viruses from rhesus monkeys as vectors for human gene therapy. *Proc Natl Acad Sci USA*. 2002; 99:11854–11859. [PubMed: 12192090]
- [20]. Mays LE, Vandenberghe LH, Xiao R, Bell P, Nam H-J, Agbandje-Mckenna M, et al. Adeno-associated virus capsid structure drives CD4-dependent CD8+ T cell response to vector encoded proteins. *The Journal of Immunology*. 2009; 182:6051–6060. [PubMed: 19414756]
- [21]. Burnett JR, Hooper AJ. Alipogene tiparvovec, an adeno-associated virus encoding the Ser(447)X variant of the human lipoprotein lipase gene for the treatment of patients with lipoprotein lipase deficiency. *Curr Opin Mol Ther*. 2009; 11:681–691. [PubMed: 20072945]
- [22]. Nathwani AC, Reiss UM, Tuddenham EGD, Rosales C, Chowdary P, McIntosh J, et al. Long-term safety and efficacy of factor IX gene therapy in hemophilia B. *N Engl J Med*. 2014; 371:1994–2004. [PubMed: 25409372] **This paper describes an important clinical success of AAV8 as a vector for gene transfer in humans and describes the long-term expression of factor IX by AAV8 for the treatment of hemophilia.
- [23]. Kou J, Kim H, Pattanayak A, Song M, Lim J-E, Taguchi H, et al. Anti-Amyloid- β Single-Chain Antibody Brain Delivery Via AAV Reduces Amyloid Load But May Increase Cerebral Hemorrhages in an Alzheimer's Disease Mouse Model. *J. Alzheimers Dis*. 2011; 27:23–38. [PubMed: 21709371]
- [24]. Wang Y-J, Gao C-Y, Yang M, Liu X-H, Sun Y, Pollard A, et al. Intramuscular delivery of a single chain antibody gene prevents brain A β deposition and cognitive impairment in a mouse model of Alzheimer's disease. *Brain Behav. Immun*. 2010; 24:1281–1293. [PubMed: 20595065]
- [25]. Patel P, Kriz J, Gravel M, Soucy G, Bareil C, Gravel C, et al. Adeno-associated virus-mediated delivery of a recombinant single-chain antibody against misfolded superoxide dismutase for treatment of amyotrophic lateral sclerosis. *Mol Ther*. 2014; 22:498–510. [PubMed: 24394188] *This is the only paper detailing the effective treatment of ALS in a mouse model using AAV-vectored antibodies to mutant SOD1.
- [26]. Wuertzer CA, Sullivan MA, Qiu X, Federoff HJ. CNS delivery of vectored prion-specific single-chain antibodies delays disease onset. *Mol Ther*. 2008; 16:481–486. [PubMed: 18180775]
- [27]. Fang J, Qian J-J, Yi S, Harding TC, Tu GH, VanRoey M, et al. Stable antibody expression at therapeutic levels using the 2A peptide. *Nature Biotechnology*. 2005; 23:584–590.

- [28]. Fang J, Yi S, Simmons A, Tu GH, Nguyen M, Harding TC, et al. An Antibody Delivery System for Regulated Expression of Therapeutic Levels of Monoclonal Antibodies In Vivo. *Mol Ther*. 2007; 15:1153–1159. [PubMed: 17375065]
- [29]. Lewis AD, Chen R, Montefiori DC, Johnson PR, Clark KR. Generation of Neutralizing Activity against Human Immunodeficiency Virus Type 1 in Serum by Antibody Gene Transfer. *Journal of Virology*. 2002; 76:8769–8775. [PubMed: 12163597]
- [30]. Johnson PR, Schnepf BC, Zhang J, Connell MJ, Greene SM, Yuste E, et al. Vector-mediated gene transfer engenders long-lived neutralizing activity and protection against SIV infection in monkeys. *Nature Publishing Group*. 2009; 15:901–906.
- [31]. Balazs AB, Chen J, Hong CM, Rao DS, Yang L, Baltimore D. Antibody-based protection against HIV infection by vectored immunoprophylaxis. *Nature*. 2011; 481:81–84. [PubMed: 22139420]
- [32]. Balazs AB, Ouyang Y, Hong CM, Chen J, Nguyen SM, Rao DS, et al. Vectored immunoprophylaxis protects humanized mice from mucosal HIV transmission. *Nature Medicine*. 2014; 20:296–300. **This study is the first demonstration that vectored delivery of bNAbs can prevent intravaginal HIV transmission. It is also the first to report the use of transmitted molecular founder strains of HIV in a humanized mouse model.
- [33]. Horwitz JA, Halper-Stromberg A, Mouquet H, Gitlin AD, Tretiakova A, Eisenreich TR, et al. HIV-1 suppression and durable control by combining single broadly neutralizing antibodies and antiretroviral drugs in humanized mice. *Proc Natl Acad Sci USA*. 2013 **This is the first demonstration that a single bNAb can sustain control of HAART-suppressed viral replication in a humanized mouse.
- [34]. Gardner MR, Kattenhorn LM, Kondur HR, von Schaewen M, Dorfman T, Chiang JJ, et al. AAV-expressed eCD4-Ig provides durable protection from multiple SHIV challenges. *Nature*. 2015; 519:87–91. [PubMed: 25707797] **This study developed a novel synthetic fusion protein that could be delivered by AAV to protect rhesus macaques from several SHIV challenges demonstrating the potential for synthetic proteins as HIV-1 vaccines.
- [35]. Deal C, Balazs AB, Espinosa DA, Zavala F, Baltimore D, Ketner G. Vectored antibody gene delivery protects against *Plasmodium falciparum* sporozoite challenge in mice. *Proc Natl Acad Sci USA*. 2014; 111:12528–12532. [PubMed: 25114213] ** This study is the first and only demonstration that vectored delivery of bNAbs can prevent malaria in a mouse model of *Plasmodium falciparum* infection.
- [36]. de Jong YP, Dorner M, Mommersteeg MC, Xiao JW, Balazs AB, Robbins JB, et al. Broadly neutralizing antibodies abrogate established hepatitis C virus infection. *Sci Transl Med*. 2014; 6:254ra129. **This is the first study demonstrating that VIP-mediated HCV specific bNAb expression can confer protection and abrogate ongoing infection in a mouse model.
- [37]. Balazs AB, Bloom JD, Hong CM, Rao DS, Baltimore D. Broad protection against influenza infection by vectored immunoprophylaxis in mice. *Nature Biotechnology*. 2013 **This study showed that AAV-vectored delivery of HA stalk bNAbs can prevent infections from multiple HA strains in both immunocompetent and immunodeficient mice. Importantly, it also demonstrated that expression of bNAb does not inhibit the elicitation of a *de novo* immune response.
- [38]. Limberis MP, Adam VS, Wong G, Gren J, Kobasa D, Ross TM, et al. Intranasal antibody gene transfer in mice and ferrets elicits broad protection against pandemic influenza. *Sci Transl Med*. 2013; 5:187ra72. *This study demonstrated the use of AAV9 to transduce nasal epithelia cells for a more transient expression of bNAb against influenza at the site of infection.
- [39]. Zhou C, Emadi S, Sierks MR, Messer A. A human single-chain Fv intrabody blocks aberrant cellular effects of overexpressed alpha-synuclein. *Mol Ther*. 2004; 10:1023–1031. [PubMed: 15564134]
- [40]. Emadi S, Barkhordarian H, Wang MS, Schulz P, Sierks MR. Isolation of a human single chain antibody fragment against oligomeric alpha-synuclein that inhibits aggregation and prevents alpha-synuclein-induced toxicity. *J. Mol. Biol*. 2007; 368:1132–1144. [PubMed: 17391701]
- [41]. Lecerf JM, Shirley TL, Zhu Q, Kazantsev A, Amersdorfer P, Housman DE, et al. Human single-chain Fv intrabodies counteract in situ huntingtin aggregation in cellular models of Huntington's disease. *Proc Natl Acad Sci USA*. 2001; 98:4764–4769. [PubMed: 11296304]
- [42]. Messer A, Joshi SN. Intrabodies as neuroprotective therapeutics. *Neurotherapeutics*. 2013; 10:447–458. [PubMed: 23649691]

- [43]. Bowers WJ, Breakefield XO, Sena-Esteves M. Genetic therapy for the nervous system. *Human Molecular Genetics*. 2011; 20:R28–R41. [PubMed: 21429918]
- [44]. Butler DC, McLear JA, Messer A. Engineered antibody therapies to counteract mutant huntingtin and related toxic intracellular proteins. *Prog. Neurobiol.* 2012; 97:190–204. [PubMed: 22120646]
- [45]. Southwell AL, Ko J, Patterson PH. Intrabody gene therapy ameliorates motor, cognitive, and neuropathological symptoms in multiple mouse models of Huntington's disease. *J. Neurosci.* 2009; 29:13589–13602. [PubMed: 19864571]
- [46]. Snyder-Keller A, McLear JA, Hathorn T, Messer A. Early or late-stage anti-N-terminal Huntingtin intrabody gene therapy reduces pathological features in B6. HDR6/1 mice. *Journal of Neuropathology and Experimental Neurology*. 2010; 69:1078. [PubMed: 20838238]
- [47]. DeMattos RB, Bales KR, Cummins DJ, Dodart JC, Paul SM, Holtzman DM. Peripheral anti-A beta antibody alters CNS and plasma A beta clearance and decreases brain A beta burden in a mouse model of Alzheimer's disease. *Proc Natl Acad Sci USA*. 2001; 98:8850–8855. [PubMed: 11438712]
- [48]. Ryan DA, Mastrangelo MA, Narrow WC, Sullivan MA, Federoff HJ, Bowers WJ. Abeta-directed single-chain antibody delivery via a serotype-1 AAV vector improves learning behavior and pathology in Alzheimer's disease mice. *Mol Ther.* 2010; 18:1471–1481. [PubMed: 20551911]
- [49]. Wang Y-J, Pollard A, Zhong J-H, Dong X-Y, Wu X-B, Zhou H-D, et al. Intramuscular delivery of a single chain antibody gene reduces brain Abeta burden in a mouse model of Alzheimer's disease. *Neurobiol. Aging*. 2009; 30:364–376. [PubMed: 17686552]
- [50]. Shimada M, Abe S, Takahashi T, Shiozaki K, Okuda M, Mizukami H, et al. Prophylaxis and treatment of Alzheimer's disease by delivery of an adeno-associated virus encoding a monoclonal antibody targeting the amyloid Beta protein. *PLoS ONE*. 2013; 8:e57606. [PubMed: 23555563] *This study demonstrated that vector-mediated antibody gene delivery was effective both prophylactically and therapeutically at reducing Aβ levels in the brains of mice.
- [51]. Lyons TJ, Liu H, Goto JJ, Nersissian A, Roe JA, Graden JA, et al. Mutations in copper-zinc superoxide dismutase that cause amyotrophic lateral sclerosis alter the zinc binding site and the redox behavior of the protein. *Proc Natl Acad Sci USA*. 1996; 93:12240–12244. [PubMed: 8901564]
- [52]. Moda F, Vimercati C, Campagnani I, Ruggerone M, Giaccone G, Morbin M, et al. Brain delivery of AAV9 expressing an anti-PrP monovalent antibody delays prion disease in mice. *Prion*. 2012; 6:383–390. [PubMed: 22842862]
- [53]. Leyland-Jones B, Arnold A, Gelmon K, Verma S, Ayoub JP, Seidman A, et al. Pharmacologic insights into the future of trastuzumab. *Ann. Oncol.* 2001; 12(Suppl 1):S43–7. [PubMed: 11521721]
- [54]. Wang G, Qiu J, Wang R, Krause A, Boyer JL, Hackett NR, et al. Persistent expression of biologically active anti-HER2 antibody by AAVrh.10-mediated gene transfer. *Cancer Gene Ther.* 2010; 17:559–570. [PubMed: 20448672]
- [55]. Ishida T, Ueda R. CCR4 as a novel molecular target for immunotherapy of cancer. *Cancer Sci.* 2006; 97:1139–1146. [PubMed: 16952304]
- [56]. Han T, Abdel-Motal UM, Chang D-K, Sui J, Muvaffak A, Campbell J, et al. Human anti-CCR4 minibody gene transfer for the treatment of cutaneous T-cell lymphoma. *PLoS ONE*. 2012; 7:e44455. [PubMed: 22973452]
- [57]. Hicks MJ, Rosenberg JB, De BP, Pagovich OE, Young CN, Qiu J-P, et al. AAV-Directed Persistent Expression of a Gene Encoding Anti-Nicotine Antibody for Smoking Cessation. *Sci Transl Med.* 2012; 4:140ra87–140ra87.
- [58]. Rosenberg JB, Hicks MJ, De BP, Pagovich O, Frenk E, Janda KD, et al. AAVrh.10-Mediated Expression of an Anti-Cocaine Antibody Mediates Persistent Passive Immunization That Suppresses Cocaine-Induced Behavior. *Hum Gene Ther.* 2012; 23:451–459. [PubMed: 22486244]

Key Points

Vectored antibody delivery represents a new strategy for the production of specific monoclonal antibodies *in vivo*.

Broadly neutralizing antibodies have been shown to prevent HIV, IAV, HCV, RSV, malaria and anthrax infection in various animal models.

Vectored antibody delivery has the potential to be used as a therapeutic for neurodegenerative diseases, cancer and substance abuse.

This approach represents an alternative vaccine strategy for vulnerable populations that cannot respond to vaccines or for diseases that are able to evade immunity.

Table 1

Summary of Monoclonal Antibody Expression Vectors

	Vector	Ab Type	Species of Fc	Isotype	Promoter	Furin/2A Cleavage	Animal Model	Vector Dose	Route	Ab Clone	Peak Serum Concentration	References
HIV	plasmid	Fab	mouse	IgG1	CMV	N/A	BALB/c mice	25 µg	IM	VRC01	3.3 µg/mL	Muthumani et al 2013
	Lentivirus	Full-length	human	IgG1	human µ heavy chain	Y	NSG humanized mice	MOI 1000	<i>ex vivo</i> B cell transduction	b12	>1 µg/mL	Luo et al 2009
	Lentivirus	Full-length	human	IgG1	hPGK	Y	NSG humanized mice	100 ng p24	<i>ex vivo</i> B cell transduction	2G12	40 ng/mL	Joseph et al 2010
	rAAV2	Full-length	human	IgG1	CMV, EF1α	N	Rag-1 mice	5×10 ¹¹ GC	IM	b12	8 µg/mL	Lewis et al 2002
	scAAV2	Immunoadhesin	rhesus	IgG2	CMV	N/A	Rhesus macaques	2×10 ¹³ GC	IM	4L6 5L7	400-500 µg/mL 200-300 µg/mL	Johnson et al 2009
	rAAV8	Full-length	human	IgG1	CASI	Y	NSG humanized mice	1×10 ¹¹ GC	IM	b12 2G12 2F5 VRC01 4E10	100 µg/mL >250 µg/mL 20 µg/mL >250 µg/mL 20 µg/mL	Balazs et al 2012
	rAAV8	Full-length	human	IgG1	CASI	Y	NSG and BLT humanized mice	1×10 ¹¹ GC	IM	b12 VRC01 VRC07 3BNC117 12A12 VRC-PG04	115 µg/mL 100 µg/mL 130 µg/mL 24 µg/mL 23 µg/mL 30 µg/mL	Balazs et al 2014
										VRC07G54W NIH45-46G54W	74 µg/mL 40 µg/mL	
										PGT121 PGT128	256 µg/mL 50 µg/mL	
										PG9	390 µg/mL	
	rAAV8	Full-length	human	IgG1	human thyroglobulin	Y	NSG humanized mice	2.5×10 ¹¹ GC 2.0×10 ¹¹ GC 2.5×10 ¹³ particles	IV IV	10-1074 3BNC117 eCD4-Ig 3BNC117	300 µg/mL 20 µg/mL 75-180 µg/mL	Horwitz et al 2013
	scAAV1	Full-length	rhesus	IgG2	CMV	N/A	Rhesus macaques	1×10 ¹³ particles	M	PGT121 10-1074 NIH45-46	-- -- --	Gardner et al 2015
Influenza	Ad5	Single-domain	--	--	CMV	N	BALB/c	1×10 ⁸ PFU 1×10 ⁷ PFU 1×10 ⁶ PFU	Intranasal	aHA-7	--	Tutykhina et al 2013

Vector	Ab Type	Species of Fc	Isotype	Promoter	Furin/2A Cleavage	Animal Model	Vector Dose	Route	Ab Clone	Peak Serum Concentration	References
rAAV8	Full-length	human	IgG1	CASI	Y	BALB/c and NSG mice, ferrets	1×10 ¹¹ GC (mice) 5×10 ¹² GC/kg (ferret)	IM	F10 CR6261	200 µg/mL (mice) 10-100 µg/mL	Balazs et al 2013
rAAV9	Immunoadhesin	human	IgG1	CAG	N/A	BALB/c mice, ferret and rhesus macaques	1×10 ¹¹ GC (mice) 1×10 ¹² GC (ferret) 1×10 ¹³ GC (rhesus)	Intranasal	F16	0.5 µg/mL (nose), 2.0 µg/mL(lung)	Limberis et al 2013
Malaria	rAAV8	human	IgG1	CASI	Y	C57BL/6NCr	1×10 ¹¹ GC	IM	2A10 2C11	>1000 µg/mL 100-500 µg/mL	Deal et al 2014
HCV	rAAV9	human	IgG1	CASI	Y	Rosa26-Fluc, FNRG humanized mice	1×10 ¹¹ GC	IM	AR3A AR3B AR4A	1000-3000 µg/mL	de Jong et al 2014
RSV	Ad5 rAAVrh.10	mouse	IgG1	CMV	N Y	BALB/c mice	5×10 ¹⁰ pfU 1×10 ¹¹ GC	IV Intrapleural	murine palivizumab precursor	--	Skaricic et al 2008
Anthrax	Ad5 rAAVrh.10	mouse	IgG1	CMV	N Y	C57BL/6 mice	1×10 ¹¹ pfU 1×10 ¹¹ GC	IV Intrapleural	14B7-1H	--	De et al 2008
HD	rAAV2	--	--	CBA	N/A	C57BL/6, BACHD, R6/2, N171-82Q and YAC128 mice	1×10 ¹⁰ GC	Intrastriatal	VL12.3 Happ1	--	Southwell et al 2009
	rAAV1	--	--	--	N/A	B6.HDR6/1 mice	2×10 ¹⁰ GC	Intrastriatal	C4	--	Snyder-Keller 2010
	rAAV1 rAAV1	--	--	CMV	N/A	3xTg-AD mice	1×10 ⁹ GC	Hippocampal	Aβ-scFv	--	Ryan et al 2010
AD	rAAV2 rAAV5	--	--	CAG	N/A	C57BL/6 and TgAβPPswε/PS1dE9 mice	3×10 ¹⁰ GC	intracranial	scFv59	--	Kou et al 2011
	rAAV2	--	--	CMV	N/A	APPswε/PS1dE9 mice	5×10 ¹⁰ GC	IM, Intraventricular	scFv	--	Wang et al 2010, 2009
	rAAV1	--	--	CMV	Y	Tg2576 mice	3×10 ¹⁰ GC	IM	IIA2	300 µg/mL	Shimada et al 2013
ALS	scAAV1	--	--	CMV	N/A	SOD1 ^{G93A} , GAP-43-luc/gfp/ SOD1 ^{G93A} mice	3×10 ⁹ GC	Intrathecal	D3H5	--	Patel et al 2014
Prion	rAAV9	-- --	-- --	--	N/A	CD1 mice	1.4×10 ¹² GC	Intracranial	D18 D18	--	Moda et al 2012

Vector	Ab Type	Species of Fc	Isotype	Promoter	Furin/2A Cleavage	Animal Model	Vector Dose	Route	Ab Clone	Peak Serum Concentration	References
rAAV2	scFv	-- -- --	-- -- --	CMV	N/A	mice	9×10 ⁹ GC	Intracranial	3:3 6:4 6:6	--	Wuertzer et al 2008
Cancer	rAAV8 Full-length	rat	IgG1	CAG	Y	NCr nu/nu mice	2×10 ¹¹ GC	IV	DC101	>8000 µg/mL	Fang et al 2005
	rAAV8 Full-length	rat	IgG1	ZgIL-2P	Y	C57BL/6 mice	2.5×10 ¹¹ GC	IV	DC101	>1000 µg/mL	Fang et al 2007
	rAAVrh.10 Full-length	mouse	IgG1	CMV	Y	C57BL/6 mice	1×10 ¹¹ GC	IV	4D5	30-40 µg/mL	Wang et al 2010
	rAAV8 scFv	humanized	IgG1	CMV	N/A	SCID-BEIGE	2×10 ¹¹ GC	IV	h1567	96 µg/mL	Han et al 2012
Addiction	rAAVrh.10 Full-length	--	--	CAG	Y	BALB/c	1×10 ¹¹ GC	IV	GNC92H2	--	Rosenberg et al 2012
	rAAVrh.10 Full-length	--	--	CAG	Y	C57BL/6 mice	1×10 ¹¹ GC	IV	NIC9D9	1300 µg/mL	Hicks et al 2012

Abbreviations: rAAV, recombinant aden-associated virus; scAAV, self-complementary adeno-associated virus; Ad5, adenovirus serotype 5; fragment-antigen binding; scFv, single-chain variable fragment; CMV, cytomegalovirus; hPGK, human phosphoglycerate kinase promoter; EF1α, elongation factor 1α; N/A, not applicable; MOI, multiplicity of infection; GC, genome copy; PFU, plaque forming unit; IM, intramuscular; IV, intravenous