

Recombinant adeno-associated virus as vaccine delivery vehicles

Review Article

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Abbreviations: Adeno-associated virus, (AAV); adenovirus, (AdV); antigen presenting cells, (APC); dendritic cells, (DCs); hemagglutinin, (HA); Herpes simplex virus, (HSV); human immunodeficiency virus, (HIV); human papillomavirus, (HPV); Intramuscular, (IM); Intranasal, (IN); intraperitoneally, (IP); Intravenous, (IV); inverted terminal repeats, (ITRs); open reading frames, (ORFs); recombinant adeno-associated virus, (rAAV); severe acute respiratory syndrome, (SARS); single stranded DNA, (ssDNA)

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Summary

Adeno-associated viruses (AAV) are non-enveloped, replication defective, single-stranded DNA virus and require co-infection with a helper virus, such as adenovirus or herpes virus, to undergo a productive infection. The AAV genome is 4.7 kb in size and is framed by two inverted terminal repeats (ITRs) at both ends of the DNA strand, and contains two open reading frames (ORFs): *Rep* and *Cap*. In total, 11 strains of AAV have been isolated and characterized from humans and primates, and new serotypes are continuously discovered. All serotypes share similar structure, genome size and organization. Serotype 2 (AAV2) has been the extensively studied and presents natural tropism towards skeletal muscles, neurons, retinal cells, vascular smooth muscle cells and hepatocytes. The most divergent serotype is AAV5 with notable differences at the level of the ITR size. AAV's natural defectiveness, its lack of pathogenicity, and the ability to infect cells *in vivo* have led to the study of its potential use as a gene therapy vector. Recent studies have begun to test AAV vectors as vaccine carriers against human immunodeficiency virus (HIV), human papillomavirus (HPV), hepatitis, severe acute respiratory syndrome (SARS), and many other viruses. rAAV vectors can evade the immune response and mediate a durable expression of transgene *in vivo*. However, evidence has been gathering that in some circumstances, the rAAV vector may initiate a cellular and humoral response to the expressed gene product *in vivo*. It is therefore important to understand the factors, which influence the establishment of these immune responses in order to design safe and efficient procedures for AAV-based gene therapies or vaccine delivery. Various factors seem to influence the immune response of AAV vaccine vectors. These include the AAV serotype, the transgene, route of administration, dose of vector, transgene expression levels, immune responses to the viral capsid, and others. A more thorough understanding of the interplay between rAAV and their encoded transgenes and the host immune system is necessary for the optimal development of rAAV vaccine system.

I. Introduction

A. AAV genome structure

Adeno-associated virus (AAV) is the smallest of known human viruses. AAV are non-enveloped, single-stranded (ss) DNA viruses with a diameter of 18-25 nm.

The virus particle is composed of an icosahedric capsid and one single molecule of the viral genome of either positive- or negative-sense. They belong to the *Parvoviridae* family and are classified in the Dependovirus genus. AAVs are replication defective and require co-infection with a helper virus, such as

adenovirus or herpes virus, to undergo a productive infection in the cultured cells. These common human viruses are naturally defective and non-pathogenic. AAVs are very resistant to extreme conditions of pH, detergent and temperature, making them easy to manipulate. AAVs are frequently found in the human populations, 70-80% of individuals having been exposed to an infectious event. So far there are no diseases associated with AAV. The virus causes only a very mild innate immune response and can infect non-dividing cells. The wild-type virus integrates into the host cell's genome, but there is no evidence that it causes malignant transformation. Because of these features it presents a very attractive choice for creating vectors for gene therapy and drug delivery.

The single-stranded DNA genome of AAV is 4.7 kb and is framed by two inverted terminal repeats (ITRs) at the ends of the DNA strand, and two open reading frames (ORFs): *Rep* and *Cap* (Figure 1). The ITRs are base-paired hairpin structures of 145 nucleotides length. They were named so because of their symmetry, which is required for efficient multiplication of the AAV genome (Bohenzky, 1988). Their ability to form a hairpin contributes to self-priming that allows primase-independent synthesis of the second DNA strand. The ITRs were also shown to be required for both integration of the AAV DNA into the host cell genome (19th chromosome in humans) and rescue from it (Weitzman et al, 1994; Wang et al, 1995) as well as for efficient encapsidation of the AAV DNA combined with generation of a fully-assembled, deoxyribonuclease-resistant AAV particles (Zhou and Muzyczckz, 1998). The ITRs contain the only necessary regulatory *cis* acting sequences required by the virus to complete its life cycle, namely the origin of replication of the genome, the terminal resolution site and, the packaging and the integration signals. In this context, Young and colleagues have shown in 2000 that

the complete ITRs are not required for integration of AAV or plasmid into the chromosome 19 site.

The left ORF or *Rep* is composed of four overlapping genes encoding four regulatory Rep proteins called Rep78, 68, 52 and 40 (Figure 1). The two major Rep proteins, Rep78 and Rep68, are involved in viral genome excision, rescue, replication and integration (Weitzman et al, 1994) and also regulate gene expression from AAV and heterologous promoters (Horer et al, 1995; Pereira et al, 1997). The minor Rep proteins, Rep52 and Rep40, are involved in replicated ssDNA genome accumulation and packaging (King, 2001). The right ORF or *Cap* is initiated at the p40 promoter and encodes the 3 structural proteins VP1, VP2 and VP3 which interact together to form a capsid of an icosahedral symmetry (Carter, 2000). Finally, all the transcripts share the same polyadenylation signal and equal amounts of virions are found containing strands of plus or minus polarity. Once inside the cell, the genome is converted to double-stranded transcriptionally active DNA, which is stabilized as a predominantly non-integrated episomal form (Duan et al, 1998; Nakai et al, 2001).

B. AAV infection cycle

The AAV infection cycle involves various steps from infecting the cell to producing new infectious particles (Ding et al, 2005; Kwon and Schaffer, 2008) including: (1) viral binding to a receptor, (2) endocytosis of the virus, (3) intracellular trafficking of the virus through endosomes, (4) endosomal escape of the virus, (5) intracellular trafficking of the virus to the nucleus and nuclear import, (6) viral uncoating, and (7) viral genome conversion from a single-stranded to a double-stranded genome capable of expressing an encoded gene (Figure 2).

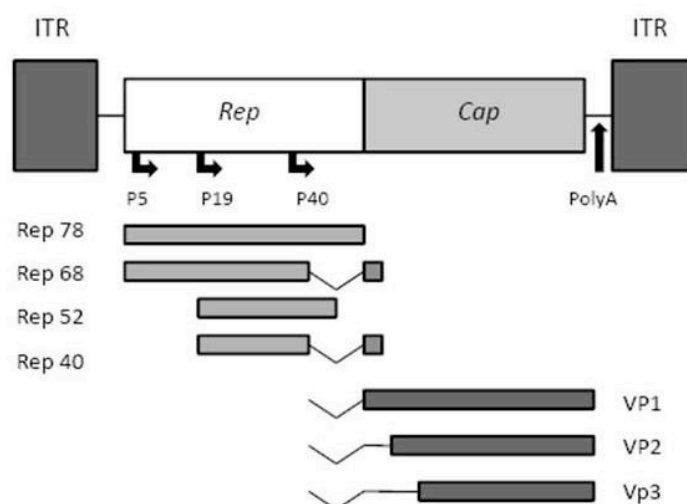


Figure 1. AAV genome organization. The transcripts for Rep78 and Rep68 are initiated at the p5 promoter. The p19 promoter produces Rep40 in spliced form and Rep52 in unspliced form. Messenger RNAs encoding the capsid proteins VP1, VP2 and VP3 are transcribed from the p40 promoter. *ITR*, inverted terminal repeats. *Poly A*, polyadenylation site. Reproduced from Merten et al, 2005 with kind permission from Gene Therapy.

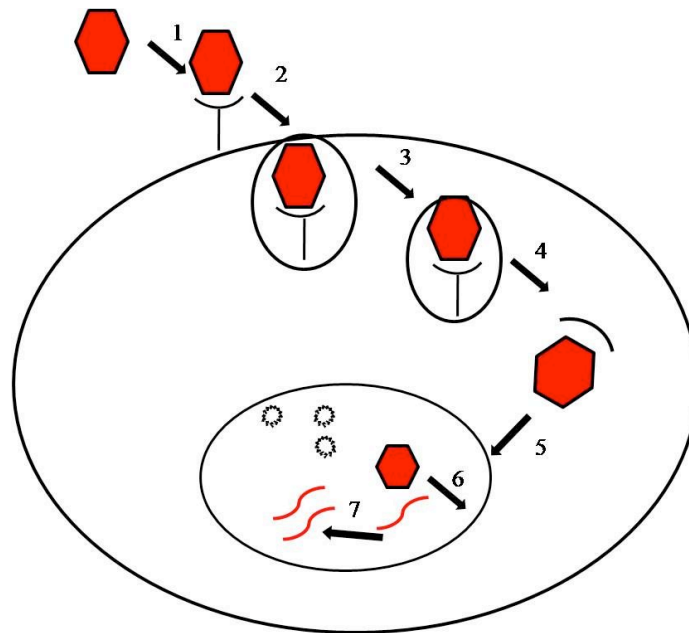


Figure 2. rAAV transduction. The various steps involved are: (1) viral binding to a receptor, (2) endocytosis of the virus, (3) intracellular trafficking of the virus through endosomes, (4) endosomal escape of the virus, (5) intracellular trafficking of the virus to the nucleus and nuclear import, (6) virion uncoating, and (7) viral genome conversion from a single-stranded to a double-stranded genome capable of expressing an encoded gene. Reproduced from Ding et al, 2005 with kind permission from Gene Therapy.

Some of these steps may look different in various types of cells, which in part, contributes to the defined and quite limited native tropism of AAV. Replication of the virus can also vary in one cell type, depending on the cell's current cycle phase (Rohr et al, 2002).

The characteristic feature of AAV is its deficiency in replication and thus the inability to multiply in unaffected cells. The first factor ascribed as providing successful generation of new AAV particles, was the adenovirus, from which the name AAV originated. It has been shown that AAV replication can be facilitated by selected proteins from the adenovirus genome (Matsushita et al, 1998; Myers et al, 1980), by other viruses such as the Herpes simplex virus (HSV) (Handa and Carter, 1979), or by genotoxic agents, such as UV irradiation or hydroxyurea (Yakobson et al, 1987, 1989; Yalkinoglu et al, 1988).

An important step in AAV viral production was achieved when the adenovirus (Ad) helper virus step was replaced by a plasmid construct containing a mini-Ad genome capable of propagating rAAV in the presence of AAV Rep and Cap genes (Matsushita et al 1998; Xiao et al 1998a). This discovery allowed for new production methods of recombinant AAV, which do not require adenoviral co-infection of the AAV-producing cells. ITRs and either Rep78 or Rep68 are sufficient for replication of the AAV genome in the presence of helper virus. In particular, Rep78 and Rep68 bind to specific sequence within the ITRs called the rep binding site (RBS) (McCarty et al, 1994; Ryan et al, 1996), and cleave in a site-and strand-specific manner at the terminal resolution site located 13 nucleotides upstream of the RBS (Brister and Muzyczka, 1999; Im and Muzyczka, 1989; Snyder et al, 1990). The RBS and terminal resolution site act as a

minimum origin of Rep-mediated DNA replication (Ward and Berns, 1995; Ward et al, 2001). In the absence of the helper virus, ITRs and either Rep78 or Rep68 are also sufficient to mediate the integration of the AAV genome into the host cell genome, preferentially into a site termed AAVS1 on chromosome 19 of human cells (Kotin et al, 1990, 1992; Linden et al, 1996; Surosky et al, 1997).

C. AAV serotypes

In total, 11 strains of AAV have been isolated and characterised from humans and primates, and new serotypes are continuously discovered. All serotypes share similar structure, genome size and organization, i.e. structure and location of ORFs, promoters, introns and polyadenylation site.

Serotype 2 (AAV2) has been the most extensively examined so far (Bartlett et al, 1998; Rabinowitz et al, 1999; Wu et al, 2000). AAV2 presents natural tropism towards skeletal muscles (Manno et al, 2003), neurons (Bartlett et al, 1998), vascular smooth muscle cells (Richter et al, 2000) and hepatocytes (Koeberl et al, 1997). AAV-2 based vectors use heparin sulfate proteoglycans as the primary receptor (Summerford and Samulski, 1998), a co-receptor fibroblast growth factor 1 receptor (Qing et al, 1999) and $\alpha_v\beta_5$ integrin (Summerford et al, 1999), giving access to a wide range of tissue types (Table 1).

Although AAV-2 is the most popular serotype in various AAV-based approaches, it has been shown that other serotypes can be more effective as gene delivery vectors. AAV6 for e.g., appears much better in infecting airway epithelial cells, AAV7 presents very high transduction rate of murine skeletal muscle cells (similar to AAV1 and AAV5), AAV8 is excellent in transducing

Table 1. Receptors of AAV serotypes on the cell membrane

AAV serotype	Receptors	References
AAV-1	-	-
AAV-2	Heparan sulphate proteoglycan (HSPG) ^a , Fibroblast growth factor receptor 1 (FGFR-1) ^b , hepatocyte growth factor receptor ^b , c-met ^b , Laminin receptor ^b , $\alpha_v\beta_5$ ^b , $\alpha_v\beta_3$ ^b	Qing et al, 1999; Summerford et al, 1999; Kashiwakura et al, 2005; Asokan et al, 2006; Akache et al, 2006
AAV-3	HSPG ^a , FGFR-1 ^b , Laminin receptor ^b	Handa et al, 2000; Rabinowitz et al, 2002; Akache et al, 2006; Blackburn et al, 2006
AAV-4	O-linked sialic acid ^a	Kaludov et al, 2001
AAV-5	N-linked sialic acid ^a , Platelet derived growth factor receptor- α ^b	Kaludov et al, 2001; Di Pasquale et al, 2003
AAV-6	Sialic acid ^a , HSPG ^b	Halbert et al, 2001; Seiler et al, 2006
AAV-7	-	
AAV-8	Laminin receptor ^b	Akache et al, 2006
AAV-9	Laminin receptor ^b	Akache et al, 2006
AAV-10, AAV-11, AAV-12	-	

a-Primary receptor

b- Co-receptor

hepatocytes (Halbert et al, 2001; Gao et al, 2002; Rabinowitz et al, 2004) and AAV1 and 5 were shown to be very efficient in gene delivery to vascular endothelial cells (Chen et al, 2005). AAV6, a hybrid of AAV1 and AAV2 (Rabinowitz et al, 2004), also shows lower immunogenicity than AAV2 (Halbert et al, 2001).

Serotypes can differ with respect to the receptor they are bound to e.g., AAV4 and AAV5 transduction can be inhibited by soluble sialic acids (Kaludov et al, 2001), and AAV5 was shown to enter cells via the platelet-derived growth factor receptor (Di Pasquale et al, 2003). The most divergent serotype is AAV5 with notable differences at the level of the ITR size (167 nucleotides for AAV5 compared to 143-146 for AAV1 to 4 and AAV6) and function. In addition, at the biological level, they are all dependent on the presence of a helper virus for their replication and gene expression.

II. AAV as a gene therapy vector

Overall, AAV vectors have shown an excellent safety record in humans in clinical trials. Broad tissue tropism, the ability to infect dividing and quiescent cells, and the long-term expression are attractive properties of this vector system. Using the rAAV vector system, many genes have been efficiently transferred into a number of tissues such as lung (Flotte et al, 1993; Halbert et al, 1997), muscle (Xiao et al, 1996), eye (Lewin et al, 1998), central nervous system (Kaplit et al, 1994; Peel et al, 1997), gut (During et al, 1998) and liver (Xiao et al, 1998b). AAV has been used to amend genetic and acquired human diseases such as cystic fibrosis, hemophilia, muscular dystrophy or diabetes mellitus (Kaufmann et al, 2001; Grimm and Kay, 2003; Hildinger and Auricchio, 2004; Nathwani et al, 2005). Despite reports that AAV induces only weak immune responses against the vector and the expressed transgene in gene therapy approaches (Sun et al, 2002; Bessis et al, 2004),

there is evidence that rAAV vectors also are efficient in genetic vaccination (Sun et al, 2003).

Most reports have described the durability of transgene expression in the tissues of AAV vector-infected animals and have demonstrated that the use of AAV vectors does not result in an immune response (especially a cell-mediated response) against the vector encoded transgene (Flotte et al, 1993; Xiao et al, 1996; Fisher et al, 1997). However, many people have neutralizing antibodies to AAV due to prior infection (Chirmule et al, 1999). Using different serotypes of AAV may circumvent this problem and allow effective long-term treatment by AAV-based gene therapy.

Two major hurdles remain for use of AAV-based gene therapy vectors: the small transgene capacity and the effect of neutralizing antibodies. AAV can package 4.9kb DNA, which is too small for many applications. By utilizing AAV's capacity to form concatemers, larger inserts can be split over two vectors and concomitantly, leading to transgene expression; however, efficiency is significantly reduced (Duan et al, 2000; Sun et al, 2000).

III. AAV vaccination and immune response

The recombinant adeno-associated virus (rAAV) has attracted tremendous interest as a promising vector for gene delivery. These vectors are simple, versatile and safe and successfully used for the long-term expression of therapeutic genes in animal models and patients. Furthermore, studies have demonstrated that rAAV vectors can evade the immune response and mediate a durable expression of transgene *in vivo* (Fisher et al, 1997; Xiao et al, 1997). However, evidence has been gathering that in some circumstances, the rAAV vector may initiate adaptive immune responses to the transgene product (Manning et al, 1997; Brockstedt et al, 1999; Lo et al, 1999). It is therefore important to understand the factors,

which influence the establishment of these immune responses in order to design safe and efficient procedures for AAV-based gene therapies or vaccine delivery.

Viral vectors are detected by the immune system and generate an immune response that becomes effective before the virus infects the target cells. Vectors induced immune response directed against them may be beneficial when the goal is vaccination or tumor lysis. However, in most cases the immune response is undesirable as it may eliminate the vector and the transfected cells decreasing both the intensity and the duration of transgenic protein expression. The immune response to gene therapy vectors, as with the infection with other microorganisms, involves the production of cytokines and chemokines that have detrimental effects. An adaptive immune response generally follows the innate response. It includes a humoral response characterized by production of neutralizing antibodies specific to the vector or transgene antigen and a cell-mediated response involving T cells and NK cells. Adaptive immunity not only contributes to eliminating the vectors and infected cells from the body but also results in a memory response that impedes further efforts to use the same vector or transgene.

Even though the mechanisms behind the ability of the rAAV-vectored transgenes to induce an immune response are not very clear, transduction of dendritic cells (DCs) following inoculation of rAAV may induce an immune response (Wang et al, 2004). DCs are key antigen presenting cells (APC) for regulating immune responses. Therefore, a major focus of present-day vaccine research is the genetic modification of DCs to express antigens or immunomodulatory molecules, utilizing a variety of viral and nonviral vectors, to induce antigen-specific immune responses that amend disease states such as malignancy, infection, autoimmunity, and allergy.

AAV, however, generate a weaker adaptive cell-mediated response compared to the other vectors such as adenovirus (AdV) (Bessis et al, 2004). This could be due to the low efficiency of AAVs to efficiently infect APCs such as DCs and macrophages (Bessis et al, 2004). Nonetheless, AAV vectors are able to infect immature DCs to some degree (Zhang et al, 2000). Furthermore, intramuscular injection of AAV vectors has been shown to direct local immune responses resulting in activation of CTL and B cell responses against the transgene (Sarukhan et al, 2001; Wang et al, 2005a,b). Given the relatively low innate immunity to AAV vectors, however, generating a sufficiently strong adaptive response for vaccine development may remain a challenge (Bessis et al, 2004).

In gene replacement therapy a gene that is not expressed in the patient is introduced *de novo* (Bessis et al, 2004). Therefore, CD4 T cells specific for the therapeutic gene have not been deleted in the thymus resulting in an immune response along with B cell activation producing antibodies. This leads to CD8 T cell activation and a cytotoxic response against the therapeutic gene. T cells can distinguish 'infectious non-self' from 'non-infectious self'. In the gene substitution therapy, the transgene by itself will not likely cause upregulation of co-stimulatory molecules on APCs, but the viruses used as vectors or sequences of nonhuman origin present in the plasmid

vector, when using naked DNA, can induce a strong host immune reaction (Onodera et al, 1999). Tolerance can thus be disrupted in such situations. Maintained expression of the transgene or repeated administration of the vector carrying the therapeutic gene is required to boost the immune system in such situations.

Humoral responses can also be generated by AAV vectors. Infection by the nonpathogenic AAV2 is common, and the prevalence of anti-AAV2 antibodies ranges from 35 to 80% according to the age group and geographic location (Chirmule et al, 1999; Erles et al, 1999; Moskalenko et al, 2000). Several studies have shown that anti-AAV antibodies have neutralizing effects that decrease the efficiency of *in vivo* vector infection in the liver (Halbert et al, 1997) or lungs, (Moskalenko et al, 2000) and therefore limit the chances of success with repeated administration of these vectors. Other studies, in contrast, have established that this humoral response has no influence on the efficiency of infection with the vector administered within the muscle (Fisher et al, 1997) or lungs (Beck et al, 1999). Similarly, the development of anti-AAV antibodies is minimal or nonexistent after administration of AAV into the brain (Lo et al, 1999; Mastakov et al, 2002) or retina (Anand et al, 2002). However, these studies were conducted in animals, which do not have pre-existing anti-AAV immunity, in contrast to humans. In humans, anti-AAV antibodies are found in serum and other body fluids such as joint fluids (Cottard et al, 2004) and amniotic fluid (Boyle et al, 2003).

The intensity of immune response varies with a number of factors, such as vector dose, the route of administration, the nature of transgene and host-related factors responsible for interindividual variability. AAV has been used as a gene delivery virus in various studies (Table 2). A number of investigators have pursued the use of AAV2 as a vaccine carrier. In this article we will be discussing only the immune response generated by AAV vectors expressing other viral genes.

A. AAV mediated vaccination

1. AAV mediated vaccination against human immunodeficiency virus (HIV)

rAAV vectors are being used currently in human trials as vaccine carriers for HIV-1. tgAAC09 consisting of ssDNA from Clade C HIV-1 genes for the gag, protease and part of the reverse transcriptase proteins enclosed within a rAAV2 protein capsid, was developed as an HIV vaccine (van Lunzen et al, 2007). In the initial trial, vaccination with tgAAC09 appears to be safe and well tolerated and stimulated a modest immune response against the gag protein. HIV-specific T-cell responses were observed in 20% of vaccine recipients receiving the highest dose of tgAAC09 tested; however antibody responses were not observed. Vectors based on other serotypes of AAV, most notably AAV1, are now entering trials (Pastor et al, 2007).

Various genes of HIV have been targeted and cloned for delivery into the cells using rAAV. rAAV-Fab105 vectors were produced by cloning the Fab105 expression cassette of HIV-1 into a AAV shuttle vector (Chen et al, 1996). When this vector was transduced into human

Table 2. immune response to AAV used as a vaccine vector.

Transgene	Targeted region/ gene	Immune response generated	Cell line / animal	Reference
HIV-1	Fab 105 expression cassette	The infection of several primary HIV-1 patient isolates was effectively blocked in the transduced lymphocytes	Human lymphocytes	Chen et al, 1996
HIV-1	env, tat, rev	IgG, IgA, MHC class-I CTL Cell-mediated immunity was enhanced	BALB/c mice	Xin et al, 2001
HIV-1	env	Systemic and regional immunity induced	BALB/c mice (Oral)	Xin et al, 2002
HIV-1	env by introducing ITRs from AAV to regulatory region of DNA plasmid (pITR/CMV-HIV plasmid)	Cellular immune response	BALB/c mice	Xin et al, 2003
HIV-1subtype B	gagV3 gene	Increase in CTL response but no increase in antibody level by pCI-gagV3	BALB/c mice	Liu et al, 2004
HIV-1	Gp 120	CTL and IgG	BALB/c mice	Feng et al, 2004
HIV-1	Gag	T cell response, IgG, Th2	BALB/c mice	Chikhlikar et al, 2004
HIV-1	Gag	CD8+ T cell response, B cell response	BALB/c mice	Lin et al 2007a
HIV-1	Gag	CD8+ T cell IFN- γ	BALB/c mice	Lin et al 2007b
HIV-1	env, gag, RT	T and B cell response	<i>Cynomolgus macaque</i>	Calcedo et al, 2006
SIV	rev-gag-PR- Δ RT-RRE; rev-env; RT-IN	T cell and Ab	Rhesus macaques (Indian origin)	Johnson et al, 2005
SARS-CoV	RBD	High neutralizing Ab	BALB/c mice	Du et al, 2006
SARS-CoV	RBD	Th1 and neutralizing Ab, Th2 and CTL response	BALB/c mice	Du et al, 2008
HPV16	HPV16 E7 fused to heat shock protein	Cellular response, CD4+ and CD8+ dependent CTL	C57BL/6	Liu et al, 2000
HPV16	L1	Neutralizing Ab	BALB/c (Intramuscular)	Liu et al, 2005
HPV 16	L1	Serum and mucosal Ab Cellular response	C57BL/6 mice (Intranasal)	Kuck et al, 2006
Hepatitis B virus	Surface antigen	Humoral immune response	BALB/c mice	Di et al, 2003
Hepatitis B/woodchuck hepatitis	Woodchuck IFN α	High Interferon levels	Intramuscular or intraportal	Pedro et al, 2005
Hepatitis B virus	IFN γ 1	Immunocytochemical studies	Mouse hepatocytes; BALB/c mice	Li et al, 2008
Hepatitis C virus	Full length (aa 1-190); truncated (aa 49-180)	CTL	Dendritic cells (DC)	Liu et al, 2006
Herpes simplex virus 2	Glycoprotein B (gB); glycoprotein D (gD)	gB specific , MHC class I CTL response; Ab titers to gB or gD increased over time	BALB/c mice (Intramuscular)	Manning et al, 1997
Chronic myelogenous leukemia	p210 ^{BCR-ABL} b3a2 variant fusion region	Cytotoxic CD4+/Th1; CD8+	DCs	Sun et al, 2002
Cytomegalovirus	Immediate early 1 (IE-1) and pp65 proteins	AB response, CD8 lymphocytes with Cytotoxic function	H2Kb mice	Gallez-Hawkins et al, 2004

lymphocytes, it produced and secreted the Fab105 fragments, while maintaining their normal morphology, growth rates, and responsiveness to mitogen stimulation. The infection of several primary HIV-1 patient isolates was also effectively blocked in the transduced lymphocytes.

HIV-1 vaccine, using an AAV vector expressing HIV-1 *env*, *tat*, and *rev* genes (AAV-HIV vector) was developed (Xin et al, 2001). A single injection of the AAV-HIV vector induced strong production of HIV-1-specific serum IgG and fecal secretory IgA antibodies as well as MHC class I-restricted CTL activity in BALB/c mice. The titer of HIV-1-specific serum IgG remained stable for 10 months. When the AAV-HIV vector was co-administered with AAV-IL2 vector (AAV with 0.7 kb murine interleukin 2 cDNA), the HIV-specific cell-mediated immunity was significantly enhanced. Also, boosting with the AAV-HIV vector strongly enhanced the humoral response. Furthermore, the mouse antisera neutralized an HIV-1 homologous strain, and BALB/c mice immunized via the intranasal route with an AAV vector expressing the influenza virus hemagglutinin gene showed protective immunity against homologous influenza virus challenge.

Similarly, systemic and regional immunity was induced in the mice after oral administration of a rAAV vector expressing HIV-1 *env* gene (Xin et al, 2002). This study also reported a significant reduction in viral load after an intrarectal challenge with a recombinant vaccinia virus expressing HIV *env* gene.

In order for predetermination of antibody affinity and specificity prior to "immunization" against HIV envelope protein, rAAV vector was used to deliver the gene for the human monoclonal antibody IgG1b12 to mouse muscle (Lewis et al, 2002). Significant levels of HIV-neutralizing activity were found in the sera of mice for over 6 months after a single intramuscular administration of the rAAV vector.

HIV-1 DNA vaccine and rAAV expressing gagV3 gene of HIV-1 subtype B were constructed, and BALB/c mice were immunized by a vaccination regimen consisting of consecutive priming with DNA vaccine and boosting with rAAV vaccine (Liu et al, 2004). CTL and antibody responses were measured and compared with those induced by DNA vaccine or rAAV vaccine separately. No evident increase in the antibody level induced by pCI-gagV3 combined with rAAV was observed, but there was an increased CTL response. The results indicate that HIV-1 specific cytotoxicity can be increased by immunization of BALB/c mice with a DNA vaccine combined with rAAV vaccine.

The potential of DNA vaccine immunogenicity improvement by introducing ITRs from AAV into the regulatory region of the DNA plasmid was tested (Xin et al, 2003). Mice immunized with pITR/CMV-HIV plasmid generated significantly higher HIV-specific antibody, higher cellular immune responses and lower viral loading than animals immunized with pCMV-HIV plasmid showing that AAV ITRs enhance CMV-dependent up-regulation of transgene expression and immunogenicity of the DNA vaccine.

In another study, the immune responses to an HIV-1 p55Gag vaccine encoded as a DNA chimera with the lysosomal associated membrane protein-1 (LAMP) was examined for the effect of the addition of the ITR sequences of the AAV to the DNA plasmid construct, and of packaging the LAMP/gag gene as a rAAV (Chikhlikar et al, 2004). The immune responses of mice to immunization with these constructs were examined using DNA prime/DNA boost, DNA prime/rAAV boost, and a single rAAV immunization. Immunization with the rAAV vector under the DNA prime/rAAV boost protocol resulted in sustained T cell responses and a markedly increased antibody response, predominantly of the IgG(1) isotype resulting from the activation of the Th2 subset of CD4(+) T cells, that was sustained for at least 5 months after immunization.

To study the immune effect of rAAV combined with rAdV vaccine in BALB/c mice, the codon-modified HIV-1 gp120 gene was inserted into a plasmid containing AAV and AdV separately to construct the rAAV and rAdV vaccines (Feng et al, 2004). Both rAAV and rAdV vaccine could express the gp120 gene in mice immunized with rAAV and rAdV. The mice primed with rAAV at week 0, 2 and boosted with rAdV at week 5, 14 and 20 elicited the strongest gp120 specific CTL and IgG antibody response.

Cell mediated T cell response and humoral responses were observed after intramuscular immunization with AAV2, AAV2/7 or AAV2/8 mixture, expressing HIV-1W61Dgp140 (*env*), Gag-Nef and HIV-1 RT in *Cynomolgus macaque* (Calcedo et al, 2006).

Similarly, a rAAV2 vaccine encoding simian immunodeficiency virus (SIV) elicited protective SIV-specific T cells and antibodies in macaques after a single intramuscular dose (Johnson et al, 2005). Furthermore, immunized animals were able to significantly restrict replication of a live, virulent SIV challenge.

Potential of AAV vectors based on novel AAVs as vaccine carriers were evaluated for HIV-1 gag in mice (Lin et al, 2007a). Strong immunogenicity in terms of gag CD8+ T-cell and antibody responses was demonstrated by AAV7, AAV8, and AAV9 based vectors. Likewise, Lin and colleagues observed in 2007 rAAV vectors expressing HIV-1 gag stimulated gag-specific response in BALB/c mice. However, CD8+ T cells induced by rAAV vectors failed to efficiently proliferate upon a booster immunization with an Ad vaccine vector or other vaccine modalities carrying the same transgene. Antigen derived from continued transgene expression in skeletal muscle induced an unresponsive phenotype in the activated CD8+ T cells. These results illustrate that additional modification are required to successfully develop an AAV-based vaccine, and that quantitation of T cell frequencies is not sufficient to determine the effectiveness of the vaccine vector. Rather, functionality of the induced T cells and the ability to re-activate these cells has to also be evaluated.

2. AAV mediated vaccination against Hepatitis

The Hepatitis B surface antigen (HBsAg) gene was cloned into the AAV vector pSNAV to form the recombinant pSNAV-HBsAg, which was transfected into

BHK-21 cells (Di et al, 2003). The cells infected with rAAV-HBsAg were capable of HBsAg expression, the amount of which augmented with the increase of multiplicity of infection. BALB/C mice immunized with rAAV-HBsAg produced anti-HBsAg antibodies. rAAV-HBsAg could induce a humoral immune response against HBsAg and therefore could be a promising candidate hepatitis B vaccine.

Interferon- $\alpha 2$ (IFN $\alpha 2$) is routinely used for anti-hepatitis B virus (HBV) treatment. AAV-IFN $\alpha 1$ was generated to deliver the IFN $\alpha 1$ gene into hepatocytes (Li et al, 2008). AAV-IFN $\alpha 1$ effectively transduced HBV-producing cells and mouse hepatocytes. A single dose administration of AAV-IFN $\alpha 1$ viral vector displayed prolonged transgene expression and superior antiviral effects both *in vitro* and *in vivo* suggesting that, the use of AAV-IFN $\alpha 1$ might be a potential alternative strategy for anti-HBV therapy.

Two rAAV vectors, carrying either the full length (aa 1-190) or truncated (aa 49-180) versions of chronic hepatitis C virus (HCV) core gene, were generated for targeting HCV-infected cells (Liu et al, 2006). Both AAV/core (1-190) and AAV/core (49-180) were used to transduce/load DCs. These two genetically altered DC types then stimulated anti-core CTL. The results indicated that the core (49-180) gene is an effective antigen, but has the advantage of stimulating less self-recognition. Thus, core (49-180) may be useful for further translational immunotherapy studies against HCV.

3. AAV mediated vaccination against human papillomavirus (HPV)

AAV vector based systems have been used for vaccination against HPV infections. A rAAV2 encoding a chimera between HPV 16 with an E7 oncogene (HPV16-E7) CTL epitope and a heat shock protein elicited a potent antitumor response against challenge with an E7-expressing syngenic cell line in immunocompetent mice (Liu et al, 2000). *In vitro* analysis indicated induction of both CD4- and CD8-dependent CTL activity. Moreover, studies with knockout mice with distinct T-cell deficiencies confirm that CTL-induced tumor protection was CD4 and CD8 dependent.

More recently, a prophylactic vaccination approach against HPV infections was investigated (Liu et al, 2005). The intramuscular application of a rAAV2 vaccine encoding the capsid protein L1 from HPV16, together with rAdV encoding murine granulocyte-macrophage colony-stimulating factor, led to induction of neutralizing L1 antibodies in BALB/c mice, when compared to the DNA vaccine (Liu et al, 2005). Immunohistochemistry, however, showed that the accumulation of APCs, such as macrophages and DCs, in rAAV-16L1 and L1 DNA-injected muscle fibers may be due to L1 protein expression, but not due to AAV infection. When compared to the non-infectious HPV-like particles (VLPs) L1 vaccine, however, the titers of neutralizing L1 antibodies induced by VLP were higher than those induced by rAAV-16L1. Co-vaccinating with rAAV-16L1 and AdV encoding murine GM-CSF (rAAV-16L1/rAd-mGM-CSF) induced higher levels of neutralizing L1 antibodies

compared with those of VLP. The data suggests that a single intramuscular co-injection with rAAV-16L1/rAd-mGM-CSF can achieve the same vaccine effect as a VLP vaccine requiring 3 booster injections, which is currently recognized as a prophylactic vaccine (Harro et al, 2001; Koutsky et al, 2002; Pinto et al, 2003).

In another study aiming to develop a vaccine against HPV16 infection, a single dose of rAAV5 L1h administrated intranasally to mice was sufficient to induce high titers of L1-specific serum antibodies, as well as mucosal antibodies in vaginal washes (Kuck et al, 2006). They observed that seroconversion was maintained for at least one year and also detected a cellular immune response even after 60 weeks of immunization. Furthermore, lyophilized rAAV5 L1h successfully evoked a systemic and mucosal immune response in mice.

4. AAV mediated vaccination against severe acute respiratory syndrome (SARS)

AAV vectors are also being utilized for vaccine development against other viruses such as SARS coronavirus (SARS-CoV).

A novel vaccine against SARS coronavirus was developed based on the rAAV delivery system by cloning the receptor binding domain (RBD) and evaluated in BALB/c mice (Du et al, 2006). High titers of neutralizing antibodies were observed against SARS-CoV infection after a single dose of RBD-rAAV vaccination. Two more repeated doses of the vaccination boosted the neutralizing antibody to about 5 times of the level achieved by a single dose of the immunization and the level of the antibody continued to increase for the entire duration of the experiment of 5.5 months. The immune responses and protective effects of immunization with RBD-rAAV prime/RBD-specific T cell peptide boost (RBD-Pep) were further assessed (Du et al, 2008). Compared with the RBD-rAAV prime/boost vaccination, RBD-rAAV prime/RBD-Pep boost induced similar levels of Th1 and neutralizing antibody responses that protected the vaccinated mice from subsequent SARS-CoV challenge, but stronger Th2 and CTL responses suggesting this vaccination protocol to be ideal for providing effective, broad and long-term protection against SARS-CoV infection.

5. AAV mediated vaccination against other viruses

rAAV vector encoding the p210^{BCR-ABL}b3a2 variant fusion region with flanking sequences (CWRBA) was constructed and used to express the BCR-ABL fusion region within primary human DCs, as a strategy for the immunotherapy of chronic myelogenous leukemia (CML) (Sun et al, 2002). CWRBA-transduced DCs elicited CD4+/Th1 and CD8+ responses demonstrating that the developed construct may serve as a vaccine for gene-based antigen-specific immunotherapy of CML.

A low titer, helper-free rAAV-pp65mII and rAAV-IE1 virus was used to elicit specific humoral and cellular responses to two important cytomegalovirus (CMV) antigens: the immediate-early 1 (IE-1) and pp65 proteins (Gallez-Hawkins et al, 2004). Simultaneous immunization

of both CMV proteins, using DNA vaccine priming followed by rAAV boost, induced antibody response and CD8 lymphocytes with cytotoxic function.

The utility of AAV vectors for genetic immunization of herpes virus-2 was examined by Manning and colleagues in 1997. Vectors expressing either HSV-2 glycoprotein B (gB) or glycoprotein D (gD) were constructed and injected intramuscularly in mice. Intramuscular injection of rAAV-gB induced a vigorous gB-specific, MHC class I CTL response and anti-gB antibody both 4 and 11 weeks postimmunization. The ability of rAAV-gB vaccination to prime a helper T-cell response was assessed by using a lymphoproliferation assay. The mice demonstrated gB-specific lymphoproliferation at 4 and 11 weeks postvaccination. The results also show that antibody titers to gB or gD increased over time.

Activation of hemagglutinin (HA)-specific CD4+ T cells and target cell destruction was triggered by rAAV-mediated gene transfer of the HA gene into the muscle (Sarukhan et al, 2001). Similarly, in a model of gene transfer in muscle, delivery of the influenza HA membrane protein by AAV was impaired by strong immune responses that lead to rapid rejection of the transduced fibers (Gross et al, 2003). However, injection of HA-specific CD4+CD25+ T cells from T-cell receptor-transgenic animals, along with gene transfer, down-regulated the anti-HA cytotoxic and B-lymphocyte responses and enabled persistent HA expression in muscle. Their results demonstrated that adoptive transfer of antigen-specific CD4+CD25+ regulatory T cells can be used to induce sustained transgene engraftment in solid tissues.

In another study using AAV as a vaccine delivery system against two strains of malaria, a single injection of rAAV encoding the malarial antigens MSP4 (*Plasmodium falciparum*) or MSP4/5 (*Plasmodium yoelii*) although stimulated long-term antigen-specific antibody responses after intramuscular injection, but the vaccination was not protective against infection (Logan et al, 2007).

B. Factors influencing the immune response of AAV vaccine vectors

1. AAV serotypes and their effect on immunization

Humans commonly carry neutralizing antibodies to human serotypes of AAV such as AAV2 and AAV1 as a result of natural infections (Mingozzi et al, 2007a), and such antibodies have been shown in gene therapy trials to reduce gene transfer (Manno et al, 2003; Mingozzi et al, 2007a). AAV-specific neutralizing antibodies would also be expected to reduce the potency of rAAV vaccines therefore; research is focusing towards developing vectors with AAV serotypes that will not generate neutralizing antibodies. Seroprevalence rates of neutralizing antibodies to the capsid of AAV serotypes isolated from nonhuman primates would be expected to be lower than those to human serotypes, which may provide the former with an advantage over the latter.

Occurrence of antibodies to AAV serotypes 1, 3, and 5 are common in humans, and they increase with age.

Seroepidemiological studies have demonstrated that AAV1, 2 and 3 antibodies prevalence rises steeply between the ages of 1 and 10, and reaches a peak of 60% by the age of 10 years. In contrast, antibody against AAV4, originally isolated from nonhuman primates, is detected much less frequently, with a peak incidence of about 10% between 2 and 5 years of age (Blacklow et al, 1968, 1971). AAV5, which was originally isolated from a human genital lesion, show average peak titers between 15 and 20 years of age (Georg-Fries et al, 1984). Among the AAV serotypes anti-AAV2 antibodies have potent neutralizing effects compared to other serotypes (Bessis et al, 2004). Xiao and colleagues found in 1999 neutralizing anti-AAV1 antibodies in 20% of individuals compared to 27% for anti-AAV2 in their study of 77 healthy individuals. On the other hand, neutralizing anti-AAV5 antibodies are generally not common in healthy individuals (Hildinger et al, 2001). Studies have also shown that neutralizing antibodies to AAV-7 and AAV-8 are rare in human sera (Gao et al, 2002), making them good vector candidate for humans.

AAV serotypes may differ in their affinities for different cell types. AAV-1, -7, -8, and -9 are the most efficient vector for infecting muscle, followed by AAV-5, 3, 2 and 4 (Rabinowitz et al, 2002). AAV-8 is most efficient for infecting liver cells in mice, while AAV-2, -5, -8, and -9 appear more similar in efficacy in hepatic gene transfer in larger animals (Sarkar et al 2006; Nathwani et al, 2007). Similar differences in tropism exist for other tissues and cell types.

AAV2, AAV2/7 or an AAV2/8 mixture, expressing HIV-1W61Dgp140 (*env*), Gag-Nef and HIV-1 RT were intramuscularly administered to three groups of *Cynomolgus macaque* (Calcedo et al, 2006). Peripheral blood mononuclear cells were collected at regular intervals after immunization and T cell mediated immune response was assessed over time. AAV2/7 or AAV2/8 induced a diverse T cell response towards Gag, Rt, Nef but not *env* whereas the AAV2 group displayed a strong response only towards *env* and negligible towards Gag, RT and Nef. In conclusion, a single intramuscular injection of AAV2/7 or AAV2/8 induced a diverse and stable T cell response and a quick and robust humoral response in *Cynomolgus macaque* monkeys.

The immune response induced by AAV serotype 1 to 8 vectors expressing HIV-1 *env* gp160 was evaluated in BALB/c mice (Xin et al, 2006). Higher HIV-specific humoral and cell-mediated immune responses were induced by AAV1, AAV5, AAV7, and AAV8 vectors expressing the *env* gp160 gene than those the AAV2 vector produced. The AAV5 vector induced the best responses demonstrating that the immunogenicity of AAV vectors depends on serotype tropism and that AAV5 is a better vector than other AAV serotypes. Additionally, mice injected with DCs that had been transduced *ex vivo* with an AAV5 vector expressing the gp160 gene elicited higher HIV-specific cell-mediated immune responses than did DCs transduced with AAV1 and AAV2 vectors.

A truncated *gag* open reading frame was cloned into an expression cassette driven by a cytomegalovirus promoter between AAV2 inverted terminal repeats (Lin et

al, 2007a). This vector plasmid was used to generate AAV vector particles with capsids from AAV1, AAV2, AAV5, AAV7, AAV8, and AAV9. Higher numbers of IFN- α -expressing Gag CD8⁺ T cells were observed in vectors based on AAV7, AAV 8, and AAV9 compared to AAVs based on capsids from types 1, 2, and 5, with the lowest frequencies noted for AAV2/2. Vectors were ranked by the levels of Gag antibodies obtained as tracked by B-cell responses ranked as follows: AAV2/8, AAV2/7 and AAV2/9 (equal), followed by AAV2/1, AAV2/5, and AAV2/2. Gag tetramer specific CD8⁺T cells peaked at about 3 weeks following vaccination and was highest for AAV2/8. Responses were higher with vectors based on AAV1, AAV7, and AAV8 compared to AAV2, AAV5, and AAV9, which may relate to the vectors' ability to efficiently transduce muscle cells.

2. Route of administration and vector dose are critical components for AAV-based immunization

Many studies have established that humoral and cell mediated immunity to AAV components or to transgenes varies with the route of administration of the vector. For example, hepatic gene transfer can induce immune tolerance to the transgene product by induction of regulatory CD4⁺CD25⁺ T cells and other T cell tolerance mechanisms (Dobrzynski et al, 2004; Cao et al, 2007a,b). On the other hand, muscle-directed gene transfer, resulting in transgene expression in muscle fibers following IM administration of vector, has been demonstrated to initiate a local immune response followed by a systemic response (Nathwani et al, 2001; Wang et al, 2005b). This route has thus far been the most utilized in rAAV-based vaccine studies. Other routes such as subcutaneous or administration to the respiratory tract remains to be studied in detail.

Virus doses and the route of administration substantially effect on the magnitude of the host immune response to rAAV2 transduction as demonstrated by murine models. For induction of significant neutralizing antibodies in a C57BL/6 mouse lung model, 10⁶ intrapulmonary rAAV2 particles per mouse were required. However, administration of vector at doses below this threshold dose also led to low level transgene expression (Halbert et al, 1998). In contrast, a higher amount of vector (10⁸ per mouse) is needed to generate anti-vector immune response to rAAV2 given IM to C57BL/6 mice (Manning et al, 1998). Interestingly, lower rAAV doses could transduce some cell types without induction of host antivector immune responses, suggesting that vector readministration might be successful following a lower primary dose. Neutralizing activities against IM (Chirmule et al, 2000) or IV (Xiao et al, 2000) administered rAAV2 were entirely T-cell dependent, as mice lacking functional CD4⁺ T cells did not generate neutralizing antibodies. These findings were consistent with those obtained in MHC class I and II knockout mice (Manning et al, 1998). However, rAAV2 delivered to the liver of mice or rhesus monkeys induced transient neutralizing humoral immune responses that were T cell independent (Xiao et al, 2000).

Other experiments using virus antigens or tumor associated antigens encoded within rAAV2 vectors also demonstrated that the delivery route influenced the type of host immune responses. The *env*, *tat*, and *rev* genes of HIV were inserted into rAAV2 under the control of the CMV-IE promoter. The highest titer of specific serum IgG was observed in BALB/c mice after immunization with these vectors by IM as compared to IN, IP, or subcutaneous routes. In contrast, the highest secretory IgA titer was induced by IN inoculation (Xin et al, 2001).

The impact of the route of administration of the AAV vector encoding human factor IX (hFIX) on the induction of an immune response against the vector and its xenogenic transgene product, hFIX was studied (Ge et al, 2001). Increasing doses of AAV-hFIX were administered by different routes to C57BL/6 mice. The route of delivery had a profound impact on serum hFIX levels as well as the induction of an anti-hFIX humoral immune response. Delivery of AAV-hFIX by an IM route induced an antibody response against the human FIX protein and no hFIX was detected in the serum of animals. However, this was in contrast in mice that received AAV-hFIX by intraportal vein (IPV) administration. When pre-existing neutralizing immunity to AAV was established in mice, AAV-hFIX administration by either the IM or IPV routes did not result in detectable serum hFIX. Although hFIX expression was not observed in mice with pre-existing neutralizing immunity to AAV, an anti-hFIX response was induced in all of the animals that received AAV-hFIX by the IM route. This was not observed in the preimmune mice that received AAV-hFIX by IPV administration. The results suggest that the threshold of inducing an immune response against a secreted transgene product, such as hFIX, is lower when the vector is administered by the IM route even in animals with pre-existing immunity to AAV.

The production of proinflammatory cytokines induced by adenoviral vectors depends on the vector dose, both *in vitro* (Liu et al, 2001) and *in vivo*, as does the intensity of the specific humoral anti-AAV response. In addition, high doses of the vector can induce tolerance to the transgene product, as shown with an AAV encoding human FIX and injected into mice via the intrahepatic route. Induction of tolerance seems to depend on the amount of transgene expressed, as is often the case with induced tolerance (Mingozzi et al, 2003). Low doses of vector can avoid induction of humoral immunity to the AAV capsid (Halbert et al, 1998; Manning et al, 1998).

Neutralizing antibodies were elicited with IV and IM infection in rhesus macaques with AAV2 while intranasal (IN) infection did not elicit any response demonstrating that primary and memory immune responses were dependent upon both the route of infection and the presence of helper virus (Sun et al, 2003). In contrast, IN coinfection with wild-type AAV2 and adenovirus elicited neutralizing antibodies, lymphocyte proliferative responses to AAV2, and cellular infiltration in local tissue (Hernandez et al, 1999).

A rAAV encoding woodchuck IFN α (AAV-IFN) to treat animals with chronic woodchuck hepatitis virus infection, a model of chronic hepatitis B was tested (Pedro et al, 2005). The vector was given by IP or IM route.

Long-term transgene expression in the liver of woodchucks was detected after IP administration of an AAV encoding luciferase. In contrast, in the majority of the animals that received AAV-IFN through the portal vein, the expression of IFN α was transient (30-40 days) and was associated with a significant but transient decrease in viral load.

Varying routes of vector administration thus may expose the same transgene product to differing immune pathways, which, in turn, may result in different immune presentation and responses.

3. Immune response to AAV capsid proteins

In vivo administration of AAV vectors leads to presentation of the viral capsid antigens to the B cells present within lymph nodes. This results in CD4+ T-cell activation, which in turn induces differentiation of B cells to plasma cells via cell cooperation mechanisms involving costimulatory molecules CD40-CD40L and cytokines such as IL-6 or IL-4. Antibodies produced by plasma cells are specific for viral capsid proteins; when they have neutralizing effects, these antibodies can prevent infection by the vectors during subsequent gene therapy attempts (Bessis et al, 2004).

Results from a recent clinical trial on hepatic gene transfer of AAV2 vector showed that rAAV vector transduction in humans induces a CTL response against the input capsid antigen (Manno et al, 2006; Mingozi et al, 2007b). The authors suggested that these capsid-specific CTLs eliminated rAAV transduced hepatocytes in human subjects, thereby causing a loss in transgene expression, and that the CTLs represented memory CD8+ T cells previously generated during natural infection of AAV in the presence of helper virus. To address the ability of AAV capsid-specific CTLs to eliminate rAAV-transduced cells in mice, it was demonstrated that AAV2 capsid-specific CTLs could be induced by DCs with endogenous AAV2 capsid expression or pulsed with AAV2 vectors (Li et al, 2007a). Others used adenoviral vectors to generate CTL responses to AAV capsid in mice (Li et al, 2007b). In either case, the investigators were unable to demonstrate elimination of AAV vector-transduced hepatocytes by AAV2 capsid-specific CTLs *in vivo* in mice, even though the AAV capsid can induce a measurable CTL response.

Humoral immune response to AAV capsid proteins following intramuscular injection and its impact on vector readministration was characterized by Chirmule and colleagues in 2000. Studies of mice and rhesus monkeys demonstrated the formation of neutralizing antibodies to AAV capsid proteins that persisted for over 1 year and then diminished, but this did not prevent the efficacy of vector readministration. Studies strongly suggested that the B-cell response was T cell dependent.

To understand the impact of AAV capsid-specific CD8+ T cells on AAV-mediated gene transfer, CD8+ T cell epitopes for AAV-2 and AAV-8 capsid in C57BL/6 (H-2b MHC haplotype) and BALB/c (H-2d MHC haplotype) mice were identified (Sabatino et al, 2005). Mice of both the H-2b and the H-2d haplotypes recognized epitopes on AAV-2 and AAV-8 capsids. T cells from H-

2b mice recognized an epitope that was conserved between the AAV-2 and AAV-8 capsids. Cross-reactivity of AAV-specific CD8+ T cells induced by different AAV serotypes may have important implications for gene transfer and identification of these epitopes will facilitate studies of immune responses to the AAV capsids.

IV. Conclusions

Vaccination has been achieved in numerous animal models; however, the successful use of AAV vectors as vaccines in animal models might not be easily replicated in humans, due to the ability of AAV to induce functionally impaired T cells and tolerance. For rAAV vectors, the magnitude and type of immune response is dependent on route of administration, the transgene itself and also expression levels as determined by serotype, promoter, and vector dose. A more thorough understanding of the interplay between rAAV and their encoded transgenes and the host immune system is necessary for the optimal development of a rAAV vaccine system. Therefore, efficient and successful gene delivery can be achieved by rAAV if the factors involved in generating immune responses are taken into consideration.

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