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Strategies to overcome host immunity to adenovirus vectors in vaccine development

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Abstract

The first clinical evaluations of adenovirus (Ad)-based vectors for gene therapy were initiated in the mid-1990s and led to great anticipation for future utility. However, excitement surrounding gene therapy, particularly Ad-based therapy, was diminished upon the death of Jesse Gelsinger, and recent discouraging results from the HIV vaccine STEP trial have brought efficacy and safety issues to the forefront again. Even so, Ad vectors are still considered among the safest and most effective vaccine vectors. Innate and pre-existing immunity to Ad mediate much of the acute toxicities and reduced therapeutic efficacies observed following vaccination with this vector. Thus, innovative strategies must continue to be developed to reduce Ad-specific antigenicity and immune recognition. This review provides an overview and critique of the most promising strategies, including results from preclinical trials in mice and nonhuman primates, which aim to revive the future of Ad-based vaccines.

Keywords

adenovirus vector; gene therapy; pre-existing immunity; toxicity; vaccine

Adenovirus (Ad) vectors have been widely investigated for delivery of gene therapy, despite well-publicised setbacks. In 2006, Ad vectors accounted for the majority (26%) of vectors used in human gene therapy clinical trials for infectious diseases and cancer [301]. Ad

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vectors encoding disease-specific epitopes have been used as vaccine approaches for a wide range of infectious diseases, including malaria, Ebola, SARS, *Pseudomonas* and HIV infections. Several features of Ad make it an ideal vector for systemic and mucosal delivery of encoded transgenes, including its low pathogenicity and relative safety profile, large transgene capacity, high transgene expression and ease of manipulation. Ad-based vaccines are particularly effective because high antigen-specific immune responses can be induced against encoded transgenes, providing prophylactic and therapeutic protection against transmitted diseases and cancer. However, the activation of innate immune responses and the presence of pre-existing immunity (PEI) to the most common Ad vectors infecting the human population increase the potential toxicity and can limit the efficacy of these vaccines [1-5].

Of the currently identified serotypes of Ad, human Ad serotypes 5 (Ad5) and 2 (Ad2) have been the most extensively investigated as gene therapy vectors. Ads are composed of a DNA core surrounded by a nonenveloped icosahedral protein capsid. The capsid is composed of structural trimeric hexon proteins, pentameric penton base proteins and trimeric fiber proteins that extend from the virus and are capped by a globular knob domain [2]. High-affinity binding of Ad5 and Ad2 knob domains to the Coxsackie virus and Ad receptor (CAR), expressed on the surface of many cells, allows the vectors to interact with integrins $\alpha_v\beta_3$ and $\alpha_v\beta_5$ through RGD peptides in the penton base [6-8]. Integrin binding mediates vector endocytosis via clathrin-coated pits [9]. Acidification of the endosome induces vector uncoating and endosomalysis [10,11], and the released viral capsid is transported by microtubules to the nucleus for docking and nuclear uptake by the nuclear pore complex [12-14].

Innate immune responses

Dose-dependent innate immune reactions to the capsids of these Ad vectors involving activation and/or recruitment of Kupffer cells, endothelial cells, neutrophils and splenic macrophages and dendritic cells (DCs), concomitant with production of proinflammatory cytokines and chemokines, appear mainly responsible for inducing the associated acute, and potentially lethal, toxicity as well as diminishing therapeutic targeting following systemic administration [15-26]. However, it is now becoming apparent that non-immune cells, transduced by Ad vector binding to CAR and α_v integrins, also respond to Ad by increasing the production of inflammatory cytokines and chemokines [5,27-30]. The overwhelming acute toxicity generated in response to a high dose of Ad vector is believed to be responsible for the death of Jesse Gelsinger [16].

Binding of Ad vectors to complement, pathogen-recognition receptors, erythrocytes and other blood factors has been implicated in facilitating these innate immune responses [31-34]. Opsonization of Ad5 by complement mediates extensive transduction of liver cells, and may be involved in Ad binding to neutrophils, Kupffer cells or platelets, leading to inflammatory cytokine and chemokine production and possibly thrombocytopenia [22,35-41]. Ad2 and Ad5 transduce hepatocytes via CAR-dependent binding, but binding to coagulation factor IX or factor X also mediates hepatocyte transduction *in vivo*, even by vectors unable to bind CAR. Factor VII and protein C also interact with Ad and mediate hepatocyte transduction *in vitro*, and transduction mediated by the coagulation factors occurs through binding to heparan sulfate proteoglycans and lipoprotein receptor-related protein [42,43]. Several recent reports have implicated scavenger receptors as the primary means by which Kupffer cells recognize and clear Ad vectors [37,44,45]. Scavenger receptor-binding to negative charges on the Ad capsid appears to mediate this uptake by Kupffer cells, although opsonization by complement and IgM may also be involved [37]. Of note, in contrast to previous reports, Xu *et al.* have recently reported that coagulation factors

are not required for delivery of Ad to Kupffer cells, and depletion of coagulation factors actually enhances Kupffer cell uptake of Ad5 while diminishing the transduction of hepatocytes [37,42]. Therefore, different methods may be required to prevent transduction of hepatocytes versus uptake by Kupffer cells. α-defensins, previously thought to prevent only bacterial infections, have now been found to bind Ad *in vitro* and prevent endosomalysis, a step required for Ad uncoating and productive transduction in cells [46].

In addition to liver sequestration of vectors and potential induction of acute toxicity, innate immune responses to Ad lead to the production of adaptive immune responses [19,36,47]. Indeed, PEI to Ad2 and Ad5 (Ad PEI) may be found in up to 50% of the American population and up to 95% of the population in other countries, and this Ad PEI can limit the effectiveness of Ad-based vaccinations [48-51].

Cellular adaptive immune responses

Since Ad-specific T cells are generated upon exposure to the virus, it is not surprising that $CD4^+$ memory T cells are detected in the majority of humans [52]. Of importance for the generation of vaccine immunity, the inflammatory cytokines released upon Ad administration contribute to the generation of T-cell responses against cells expressing proteins derived from the Ad vector and/or the transgene [19,53,54]. However, T-cell-mediated Ad PEI has been shown to diminish T-cell responses to transgene products [55-57]. Following cellular transduction, Ad capsid peptides or peptides from the transgene product are presented to T cells via MHC-I molecules on the surface of transduced cells. In addition, DCs endocytose Ads indirectly (by scavenging apoptotic debris from transduced cells) or directly (through nonspecific macropinocytosis or targeted receptor-mediated uptake) and can present vector-encoded peptides to T cells via both MHC-I and -II molecules, thereby activating both $CD4^+$ and $CD8^+$ T cells [58]. Furthermore, DC activation by Ads, possibly by Ad genomic interactions with nacht domain-, leucine-rich repeat-, and PYD-containing protein (NALP)3 and Toll-like receptors (TLRs) [59-64], promotes Th1-type $CD4^+$ T-cell responses to peptides derived from Ad vector genes and transgenes. While Th2-type $CD4^+$ T cells provide help to B-cell maturation and isotype switching, Th1-type $CD4^+$ T cells provide help for the development and expansion of cytotoxic $CD8^+$ T cells [65]. Thus, transduced DCs generate cytotoxic $CD8^+$ T cells capable of eliminating transduced cells expressing Ad genes or transgene-product peptides [66-73]. This is clearly beneficial in the context of mounting an effective response against the disease-specific transgene product in order to combat intracellular pathogens and tumors. However, the cytotoxic T cells generated against Ad vector proteins eliminate transduced cells expressing Ad genes as well as the transgene, resulting in diminished immunotherapeutic gene expression.

Ad-specific effector and memory T cells can be quickly activated following Ad vector administration [74]. The capsid protein hexon appears to be the major target for $CD4^+$ and $CD8^+$ T cells, although T cells have also been detected against Ad early proteins. In addition, several of the epitopes recognized by T cells are conserved among Ad serotypes, including Ads tropic for other species [74-83]. Importantly, as the E3 region of Ad normally down-regulates MHC-I expression by infected cells, Ads with an E3 deletion (which increases the transgene ‘payload’ size) may actually enhance recognition of Ad-transduced cells by cytotoxic T cells, and T cells specific for early genes could eliminate Ad-infected cells before production of the encoded transgene is underway [84-86]. As rehighlighted by results of the recent anti-HIV vaccine STEP trial, this T-cell-mediated Ad PEI can hinder effective vaccination and may even result in increased susceptibility to infection or enhanced vector-related toxicity [87-90].

Humoral adaptive immune responses

In addition to Ad-specific T cells, Ad-specific neutralizing antibodies (NAbs), predominately directed against hexon, fiber and penton base, are generated following the first exposure to Ad, whether naturally occurring or after one immunization with an Ad vector [51,91,92]. These NAbs inhibit Ad transduction of cells by a variety of methods, including prevention of cell attachment or facilitation of virus aggregation and clearance [51]. In addition, antihexon NAbs that allow virus entry but prevent virus gene expression have been identified [93-95]. One such monoclonal antihexon antibody has been characterized and shown to prevent nuclear entry by blocking microtubule-dependent intracellular transport of the virus following endosomal escape [44,96]. NAbs generated against hexon hypervariable regions (HVRs), which account for 80–95% of anti-Ad NAbs, appear to be most critical for virus clearance, and significantly limit immunotherapeutic expression of genes delivered by Ad vectors [97]. Therefore, methods that prevent the induction of anti-Ad immunity and allow Ad vectors to evade PEI including Ad-specific T cells and NAbs are critical for advancing effective Ad-based gene therapies.

A variety of such methods have shown great promise, and this review will primarily focus on the most recent advances and the effects these techniques have on avoiding both acute toxicity and adaptive immune responses. In particular, reports in which these methods have been specifically evaluated in the presence of Ad PEI will be discussed.

Immunosuppression

Critical initial investigations showed that immunosuppressive drugs enhance transgene expression by Ad vectors by preventing the development of antibody- and T-cell-mediated immunity to Ad. These studies opened the door for the development of immune-evasion techniques, but the clinical utility of immunosuppressive drugs to prevent anti-Ad immune responses was questioned owing to safety concerns [98]. Many of the pathways involved in the initial phase of acute anti-Ad immune responses have now been elucidated. However, these pathways are highly redundant, thus blockade of any one pathway is unlikely to provide complete protection against anti-Ad immunity or toxicity [60,99]. Therefore, the continued evaluation of other methods for circumventing anti-Ad immunity is required. Following previous reports that suggested pretreatment of mice with anti-inflammatory glucocorticoids prevents Ad-induced activation of some innate and adaptive immune responses and allows effective re-administration of vector, Seregin *et al.* have reported that pre-treatment of mice with dexamethasone (DEX) prevents innate immune responses to Ad5 vectors and diminishes anti-Ad adaptive responses [100,101]. Specifically, pretreatment with DEX prevented Ad5-induced release of inflammatory cytokines/chemokines, thrombocytopenia, and inflammatory gene expression and leukocyte infiltration in the liver. DEX treatment did not protect against Kupffer cell loss following Ad injection, however. Interestingly, increased levels of Ad vector genomes were detected in liver cells at early time points postinjection, but these did not translate to higher levels of transgene expression. As anticipated, following the blunting of acute immune responses to the vector, adaptive immune responses were also altered. In mice receiving DEX, not only was the generation of all Ad capsid-specific primary antibodies reduced, but Th1/Th2 responses were significantly diminished as well. This suggests the usual T-cell-mediated clearance of cells expressing Ad vector-derived peptides may be reduced, which in turn may lead to higher transgene expression levels. In addition, Ad-specific NAbs were significantly reduced. While transgene product-specific (LacZ) IgM and IgA antibodies were reduced in DEX-treated mice, relative concentrations of transgene product-specific IgG antibodies were unchanged. Only a slight reduction in the ratio of Th1/Th2 response to the transgene product, as determined by antibody isotype prevalence, was observed, suggesting that potential vaccine-

induced immunity would not be compromised. Overall, these results suggest that pretreatment with an anti-inflammatory glucocorticoid may provide protection from Ad vector-induced toxicity and generation of Ad-specific NAbs in naive animals, while preserving the potential immune response to the transgene product. Previous reports have also demonstrated enhanced transgene product expression following Ad administration *in vivo* [100,102]. Thus, it seems clear that pretreatment with DEX can provide important benefits regarding the safety of systemically administered Ad-based vaccines and may improve transgene expression, although the overall efficacy of this treatment must also be examined in animals with Ad PEI.

Ad-delivered transgenes are not considered to be efficiently incorporated into the genome of the host cells, thus Ad-mediated gene expression diminishes over time, even in the absence of Ad PEI. Therefore, repeat administration of Ad vectors is necessary for effective treatment of many diseases. In this regard, prime–boost vaccination strategies are often used to amplify the effective numbers of T cells that are required to initiate rapid responses to encoded antigens [103]. However, Ad PEI limits the induction of further transgene product-specific immune responses achieved following boosting in animal models. Therefore, as the majority of humans already have Ad PEI, it is imperative that methods for PEI evasion be developed and instituted for the future utility and success of Ad-based vaccine strategies.

Alternative serotypes & genetic modifications

Alternative serotypes

To overcome the potential limitations of Ad-based vaccines in humans with Ad PEI, alternative serotypes belonging to different subgroups have been investigated for the ability to avoid acute toxicity and evade anti-Ad immune clearance. NAbs generated against unique Ad serotypes are generally specific and do not cross-react with other serotypes [104-106]. However, many serotypes, such as Ad49, Ad35 and Ad11, do not afford the same benefits of Ad5 because they are unable to induce high transgene expression and are less amenable for large-scale production. Although initially problematic, novel strategies to overcome these obstacles are showing promise. For example, the PER.C6 complementing cell line can be used to propagate high titers of Ad49 vectors. Although transgene product-specific immune responses induced by Ad49 vectors are unaffected by Ad5 PEI, higher vector doses are required to achieve responses comparable with those generated by Ad5 vectors in naive mice, and so may prove less beneficial for immunotherapy [107].

Ad serotypes such as Ad7, Ad35, Ad40 and Ad41 vary greatly from Ad5 in fiber domains [108-110]. CAR-mediated transduction of liver cells is reduced with these vectors, and chimeric Ad5 vectors expressing the fiber and shaft domains of these serotypes have been examined for their ability to reduce liver transduction *in vivo* [108,111]. Hepatocyte transduction, inflammatory cytokine and chemokine production, and acute liver toxicity in mice are significantly diminished following administration of Ad5 vectors engineered to express the Ad35 knob and fiber [112]. Interestingly, this appears to depend on the length of the shaft domain, rather than on the ability of the knob to directly bind CAR *per se*, since Ad5 vectors expressing the knob and shaft domains of Ad9, a CAR-binding serotype with a short shaft, also reduce acute liver toxicity. This diminished toxicity is presumably due to a combination of factors resulting from the shortened shaft domain, including reduced accessibility for receptor binding as well as the inability to bind blood factors [112]. Similar decreases in the production of inflammatory cytokines and chemokines were observed with chimeric vectors expressing long and short Ad41 fibers, but lacking heat-shock protein-interacting domains [113]. Of note, while the acute production of inflammatory cytokines/chemokines was reduced, adaptive immune responses generated against the vector were similar to those generated against unmodified Ad5 vectors [113]. Further deleting the RGD

motif of these vectors reduced hepatocyte transduction and inflammatory responses even further, but these vectors also induced anti-Ad adaptive responses comparable with those of unmodified Ad5 [114].

Nonhuman adenoviruses

The development of vectors based on Ads that normally infect nonhuman species may also provide a promising solution. Indeed, chimpanzee Ads (AdC) have gained popularity since the initial demonstration that human serum does not significantly neutralize AdC vectors [115], and unlike some alternative human serotypes examined, the E1-deleted version of AdC7 is easily propagated [116]. An AdC7 vector expressing the SARS-coronavirus antigen elicited higher T- and B-cell responses than an Ad5 vector in mice with Ad5 PEI [117]; and in mice with Ad5 PEI, a single injection of AdC7 encoding the Ebola glycoprotein provided protection from lethal challenge, unlike the corresponding Ad5 vector [116]. Although Ad5 PEI did not impact the protection conferred by the AdC7 vector, it did slightly reduce anti-Ebola T- and B-cell responses [116], and similar decreased immune responses were observed after immunization with an AdC68 vector expressing HIV Gag [118]. These results suggest that although T- and B-cell responses generated against transgene products encoded by AdC vectors may be less robust in the presence of PEI, they may still be sufficient for effective therapy.

A more recent report examined the protective effects of single-dose vaccinations with three different AdC vectors, AdC6, AdC7 and AdC9/C68, encoding a fusion protein of T- and B-cell epitopes of malaria [119]. In mice with or without Ad5 PEI, AdC7 and AdC9 vectors induced greater antigen-specific T-cell responses than either Ad5 or AdC6 vectors (with no differences between intramuscular and intradermal injection). In the presence of Ad5 PEI, protection induced by all Ad-based vectors was reduced. Immunization with Ad5 offered no protection, while AdC9 offered the best protection, but only in 50% of the mice (compared with 92% without Ad5 PEI). Further studies by this group showed that the immune response and protection conferred by AdC6 could be increased by adding the full intron A sequence to the human cytomelagovirus promoter used to drive antigen expression [120]. However, these authors also point to the fact that PEI to AdC6 has been detected in some African populations, and this AdC6 PEI reduced the efficacy of AdC6 vectors [120,121]. Furthermore, as with other Ad vectors, it is likely that AdC6 PEI will be induced by exposure to the initial vaccine, and may affect the efficacy of subsequent vector immunizations. The nature of anti-AdC6 responses, with respect to their impact on AdC6 neutralization and duration of transgene product expression, remains to be investigated.

It is especially important to note that several Ad epitopes recognized by T cells are conserved among a broad array of human and nonhuman primate-derived Ads, making it likely that T cells in patients with Ad5 PEI will also recognize vectors derived from these viruses [74,80,82,83]. Bovine infectious Ads have been examined, since NAbs to bovine Ad3 (BAd3) have not been reported in human populations. In the mouse model, a single-dose injection of BAd3 vectors encoding the hemagglutinin (HA) antigen of H5N1 influenza induced greater levels of cellular immunity than Ad5 vectors, and this was not diminished by Ad5 PEI [122]. Most importantly, mice with Ad5 PEI receiving a prime–boost regimen of BAd3–Ad5 vectors encoding HA were fully protected from lethal influenza virus challenge, while those receiving a homologous regimen of Ad5–Ad5 vectors were not. Thus, Ad vectors that normally infect species other than humans may induce immune responses and offer protection comparable or superior to Ad5, and maintain protection even in the presence of Ad5 PEI. As highlighted by Kobinger *et al.*, vectors capable of affording complete protection in one dose without the need for additional boosts are critical for treating diseases that require ‘rapid intervention’ [116]. Thus, it will be worth examining the potential merits of vectors based on Ad vectors with specific tropism for other species too,

such as canine, porcine and chicken [123-125]. However, both opsonizing antibodies and reactive $CD4^+$ memory T cells specific for canine Ad2 (CAV-2) have been detected in humans, indicating that this may not be such a simple solution for evading Ad PEI [126]. These studies also emphasize that different viral vectors affect the quality of immune responses to antigens, and these responses must be thoroughly investigated in the context of human immunity to predict the efficacy of vaccines in humans. In cases where prime–boost strategies remain necessary for complete protection or disease regression, immunity generated against the priming vector dictates the need to use alternative vectors for priming. Therefore, the continued validation of alternative serotypes and chimeric vectors is critical for defining the available repertoire of vectors that may be used for prime–boost immunizations. Another consideration must be raised since Ad vectors may activate TLRs, influencing the innate immune response to the vector and the transgene product [5,60-64]. Different Ad serotypes appear to induce phenotypically and qualitatively different immune responses to the transgene product [127]. This observation is consistent with the notion that differential interaction with TLRs may contribute to this phenomenon, but this is not yet confirmed. Previous studies investigating the effects of priming with antigen in the form of either plasmid or protein followed by Ad vector-induced boosts have shown that the magnitude and quality of T-cell responses can be significantly influenced by the form of priming vaccine [128-131]. Similarly, priming with Ad vectors of different serotypes may significantly alter the humoral and cellular response to the transgene-encoded vaccine. In a recent study comparing heterologous prime–boost regimens with homologous regimens, Barouch's group found that simian immunodeficiency virus (SIV)–Gag-specific responses generated in rhesus monkeys by heterologous prime–boost immunizations (Ad26–Ad5) was greater when compared with homologous prime–boost immunizations with Ad5 vectors [132]. In addition to boosting humoral and cellular responses, heterologous vector administration increased the number of Gag-specific epitopes recognized by T cells by nearly four times. Prime–boost immunizations with Ad35–Ad5 vectors produced enhanced Gag-specific immune responses over Ad5–Ad5 vaccination, but attained only 50% of the magnitude generated by the Ad26–Ad5 strategy. Schirmbeck *et al.* further demonstrated that, in naive mice, Ad-specific T-cell responses generated after a single injection of an Ad vaccine profoundly influence the diversity of the antigen-specific T-cell response [133]. In contrast to responses following a single immunization with antigen encoded in a DNA-based vaccine (plasmid DNA-encoded transgene), Ad vector-encoded antigen was unable to generate T-cell responses to subdominant epitopes of the antigen. Thus, the efficiency with which the Ad- and transgene product-derived peptides are presented following transduction dictates the kinetics of the development of epitope-specific T-cell responses and suggests that subdominant epitopes are not effectively presented in the time it takes to induce Ad-specific cytotoxic T-cell responses. This may explain the observation that selective increases in transgene product-specific immune responses over Ad-specific T-cell responses are observed when DEX is administered in conjunction with Ad vaccination, further supporting the use of temporary immune suppression to enhance Ad vector-based vaccinations [101]. Recent results have also documented profound Ad species-related differences in the ability to stimulate secretion of IFN-α, Mip-1α and IL-6 cytokines from human plasmacytoid DCs (pDCs), an important part of the innate immune system [74]. Interestingly, this study showed that human Ad5 demonstrated no stimulatory effect on human pDCs. Thus, the combination of Ad vector sero- or species-type, plasmid DNA or protein antigen used for prime–boost strategies may provide different adjuvant signals and could be used in optimized combinations tailored to achieve protective immunization against a particular pathogen.

Hexon modifications

The discovery that the majority of Ad5 NAbs are directed against Ad hexon HVRs [97], and confirmation that the HVRs of Ad5 could be engineered to incorporate foreign peptides, provided the rationale for the developing chimeric Ad5 vectors that express the HVRs of other serotypes [134-136]. Chimeric Ad5 vectors expressing the HVRs from Ad48 (Ad5HVR48) induced significantly greater immune responses to the transgene product than unmodified Ad5 vectors did in both mice and rhesus monkeys with Ad5 PEI [137]. Ad5 chimeric vectors in which all seven HVRs were exchanged induced the same level of anti-antigen immune responses in mice with Ad5 PEI as in naive mice. However, replacing only one HVR provided little improvement over non-chimeric Ad5 vectors. The antigen-specific immune responses induced by the Ad5HVR48 vector were greater than those induced by immunization with Ad48 or Ad35 vectors, confirming the initial hypothesis that these chimeric Ad5 vectors would retain the immune-generating capacity of Ad5 vectors.

The hexon HVRs were also found to be major binding sites for factor X (and possibly for factor IX) [138,139]. The binding affinities for factor X vary between serotypes, and may explain differences in hepatocyte transduction *in vivo* previously observed between serotypes. Although some differences in binding affinities were noted in these reports, overall, Ad2 and Ad5 bound factor X with the highest affinity, while weak or no binding was detected for Ad9, Ad35, Ad48 and Ad51. Factor X binding to the Ad5HVR48 chimeric vector (described previously) was significantly reduced, as was binding to an Ad5 vector with a hexon mutation. Transgene expression in the liver was also dramatically decreased following intravenous administration of either vector in mice [138,139]. Pre-injecting mice with the snake venom-derived protein X-bp, which blocks factor X binding to Ad5, also prevented expression of transgenes in the liver [139].

A paradigm for simultaneously evading Ad immunity and generating a more robust, disease-specific immune response is based upon substituting the highly antigenic Ad-specific epitopes of capsid proteins with vaccine-specific antigen epitopes [140-143]. Such capsid-modified Ad vectors provide the vaccine antigen as a component of the capsid rather than as a transgene. Incorporation of immunogenic peptides into the Ad capsid offers potential advantages. Hypothetically, the exogenous protein source (as opposed to an endogenous source of antigen provided by transgene expression) and processing of the hexon-incorporated antigen should result in a humoral response akin to the response provoked by native Ad capsid proteins. In studies designed to determine the ideal immunogenic capsid location for epitope insertion, Krause and colleagues analyzed responses induced in mice by vaccination with Ad vectors modified by antigen epitope insertion into different capsid proteins [144]. Somewhat surprisingly, insertion of influenza HA into the fiber knob induced the strongest cellular and humoral immunity against the antigen in comparison with vaccination with vectors incorporating the antigen in hexon, penton base or pIX. However, this may also be influenced by the size and exact location of insertion.

As noted, immunodominant epitopes recognized by Ad NAbs are found in the highly abundant hexon protein, specifically in surface-exposed HVR loops, and hexon modification can prevent Ad binding to coagulation factors [137-139,145]. Worgall *et al.* advanced this approach by developing a vaccine from Ad5 vectors expressing a *Pseudomonas aeruginosa* epitope in the hexon regions [146]. This vaccine is effective against *P. aeruginosa* challenge in mice, and repeat administration of the vaccine enhanced the humoral and cellular responses to the *P. aeruginosa* epitope, although this was not specifically investigated in mice with Ad PEI. Most recently, our group further advanced this paradigm by defining a range of sites within hexon as a platform to present antigenic epitopes, or other peptide motifs, that permit the assembly of viable capsid-modified vector. These data illustrate that a range of antigenic epitopes can be inserted into hexon HVRs 2 or 5, with Ad5 HVR5

demonstrating greater permissiveness to a range of insertions that still allow production of viable Ad virions. Our results also indicate that vaccination with an Ad vector containing an antigenic peptide epitope incorporated within the Ad5 capsid hexon, in the absence of any encoded transgene product expression, induces a significant humoral response to that epitope [134,147]. It is likely that, in addition to the capsid location of antigen insertion, the efficiency of antigen processing would be highly dependent on the nature of the peptide sequence chosen. Whether capsid-modified Ads aid vaccine boosts by focusing the enhanced immune response on the incorporated antigen is currently under investigation. Given the recent information regarding factor X binding to hexon regions, this strategy may be particularly useful in instances when liver untargeting is required. In summary, these studies suggest that the 'capsid-incorporated antigen' approach may offer a highly effective and versatile vaccine strategy that could also impart some protection against Ad PEI. However, the studies by Kalyuzhiny *et al.* also pointed to a previously unobserved role for hexon in the intracellular trafficking of Ad vectors that is necessary for optimal transduction in some cell types [138]. Thus, careful consideration should be given to hexon modifications, depending on the identity of cells to be targeted.

Helper-dependent adenoviruses

Gutless, helper-dependent Ad (HD-Ad) vectors are devoid of Ad genes and consist only of the Ad packaging and inverted terminal repeat sequences. These vectors have been specifically developed as a means to avoid cellular immunity to Ad viral genes and diminish liver toxicity, and thus promote long-term transgene expression [98,148-150]. However, HD-Ad vectors are still susceptible to vector-specific NAbs and can induce dose-dependent toxicities. This was observed in nonhuman primates following systemic administration of HD-Ads, indicating that the anti-Ad responses that plague the use of unmodified Ad vectors would also limit the utility of HD-Ad vectors [151-153]. Further studies indicated that HD-Ad vectors delivered systemically induce early, capsid-dependent inflammatory cytokines similar to those induced by Ad5 vectors, although the induced cytokine release is short-lived in comparison and adaptive immune responses are not induced [154]. The discovery that the majority of anti-Ad immune responses are produced against capsid proteins further emphasizes that, similar to unmodified Ad vectors, systemically delivered HD-Ad vectors must evade capsid-specific PEI to provide optimal efficacy in humans [51,81,82,91,92].

Biochemical modifications

PEGylation

Multiple biochemical methods for masking Ad epitopes have also been investigated, which include the use of sodium alginate, poly-hydroxypropyl methacrylamide or activated polyethylene glycol (PEG) polymers [3,98,155]. Modification of proteins or viruses with PEG polymers, referred to as PEGylation, improves water solubility, is nontoxic, nonimmunogenic and can mask Ad epitopes [156-158]. Early reports showed that PEGylation of Ad5 inhibits recognition by NAbs and T cells, decreases induction of inflammatory cytokines such as IL-6, and enhances storage stability of Ad5 as well [159-166]. PEGylation also blocks CAR-mediated transduction of cells, which may reduce vector uptake by hepatocytes and diminish toxicity [164,167,168]. Furthermore, PEG molecules can be modified to retarget Ad5 vectors to bind alternative cell type-specific molecules for transduction, such as EGF receptor, FGF receptor, E-selectin and integrins [163,168-171]. However, reports vary on whether transgene product expression levels and transgene product-specific immune responses are reduced when vectors are PEGylated [160,165,172-174]. In this regard, ensuring that a majority of Ad5 capsid proteins are attached to PEG polymers may enhance transgene product expression following systemic

vector injection [158], but the size of PEG modifications appear to be critical for optimal transgene product expression as well as evasion of Ad5 PEI.

It is now apparent that differences in the molecular weight of PEG polymers used to mask the vector may account for variances in reported immune protection, CAR interaction and transgene-product expression. While vector modification with small PEG polymers of 2 and 5 kDa in size may confer immune protection, larger polymers of 20 kDa and above appear to be required to fully prevent CAR/integrin-mediated transduction and liver transgene-product expression by modified Ad5 vectors in mice [173,175,176]. These studies also corroborate previously published results showing that PEGylated vectors produce significantly lower immune responses to the encoded antigen, although this may also be influenced by the route of vector administration, as discussed below [173,176]. However, in the presence of Ad5 PEI, Ad5 vectors modified with PEG polymers of at least 20 kDa induce significantly greater transgene product-specific immune responses [173]. Interestingly, Weaver and Barry report that modification of Ad5 vectors with 5- and 35-kDa PEG polymers does not prevent the generation of anti-Ad5 NAbs following systemic injection [176]. While this study did not specifically evaluate the effects on developing Ad5-specific T cells, it does indicate that PEGylation with larger-molecular-weight polymers should be considered for maximal transgene delivery and induction of transgene product-specific cellular and humoral immune responses. In addition, further examination by Hofherr *et al*. suggests that PEGylated vectors (5 and 35 kDa) still interact with blood factors IX and X, and are thereby capable of transducing liver cells in culture at low levels [175]. Thus, it will be important to determine whether the low levels of liver cell transduction are also observed *in vivo*.

PEGylated vectors may also prove beneficial for prime–boost strategies. This was systematically investigated by Weaver and Barry [176]. They found that when unmodified Ad5 was used for both the prime and boost by intramuscular injection in naive animals, no boost response in antigen-specific antibody levels was observed. However, when PEGylated Ad5 was used for both the prime and boost, antigen-specific antibody levels were boosted, increasing the low levels of prime-induced antibody to levels attained with unmodified Ad5. Therefore, Ad5 NAbs that were detected in this study following immunization with PEGylated vectors (inducing PEI) did not significantly block the ability to boost transgene product-specific humoral responses, suggesting multiple boosts may be administered. However, transgene product-specific cellular responses were blunted when homologously, but not heterologously, PEGylated vectors (with respect to 5- or 35-kDa PEG polymer modifications) were used to induce PEI [176]. Thus, PEGylating with different size polymers may prove to be advantageous in broadening the vector repertoire that can be utilized in prime–boost schemes and in the presence of Ad PEI. This will need to be corroborated in studies with nonhuman primates and, ultimately, in clinical trials.

Poly(*N*-[2-hydroxypropyl]methacrylamide)

Covalent modification and retargeting of Ad vectors has also been achieved with poly(*N*-[2-hydroxypropyl]methacrylamide) (HPMA), which reacts with amine groups on the virus surface [177]. HPMA coats are also nontoxic and nonimmunogenic [178]. Coating of Ad5 with HPMA prevents vector binding to CAR and subsequent cell entry, and targeting ligands such as EGF, FGF and VEGF can be incorporated in the polymer coat to facilitate vector uptake and transgene product expression by targeted cells [177,179]. The inclusion of reductively degradable disulfide bonds facilitates intracellular uncoating of the HPMA polymer, allowing productive Ad5 infection [180]. A recent report also indicates that increasing polymer concentrations on the Ad surface from 2 to 20 mg/ml further prevents vector transduction of CAR-positive cells, and that the timing of the transgene product expression is not affected by HPMA coating [181]. In addition, Ad5 NAbs do not recognize

HPMA-coated vectors, allowing efficient transgene product expression *in vitro* [177,179,181]. HPMA-coated Ad5 vectors have an increased plasma half-life in mice, and association with human erythrocytes is significantly decreased *in vitro*, allowing Ad5 transduction of cells and transgene-product expression in the presence of human erythrocytes [179,182]. Interestingly, nonspecific targeting to liver is dramatically decreased when FGF-targeted HPMA-coated Ad5 is administered intravenously in mice [181].

Carlisle *et al.* have recently demonstrated that coating Ad vectors with newly designed HPMA polymers also prevents factor X-binding, unlike PEGylation [175,179]. Therefore, HPMA coating appears to provide shielding of Ad vectors from both the innate and adaptive arms of the immune systems. However, the full potential of this polymer has yet to be examined in animal models with Ad PEI. In particular, the effects of HPMA coating with regard to T-cell responses to both Ad vector proteins and transgene products should be investigated. Although factor X-binding to hexon is blocked, hexon epitopes will still be presented to T cells. In addition, if the efficacy of Ad-based vaccines relies, at least in part, on direct uptake by DCs, this may be facilitated by Ad–antibody immune complexes internalized through Fc-receptor (Fc-R) interactions. A combined technology that merges HPMA coating with the addition of targeting ligands may be important to facilitate and enhance the induction of the Ad vaccine-specific response. Targeting to DCs would be an obvious next step. Alternatively, targeting to cells via EGF or VEGF may allow targeted cells to become substantial antigen production 'factories'. Produced antigens secreted as proteins or in apoptotic bodies may be ably endocytosed by DCs for effective induction of both $CD4^+$ T cells and via cross-priming, $CD8^+$ T cells. The most effective way to express antigen genes to achieve the greatest magnitude and appropriate quality (type) of response is not completely known, and will probably require extensive investigation with respect to each individual antigen or disease.

Bispecific adapters

Alternative strategies for shielding Ad from PEI include the utilization of bispecific adapters that bind to Ad capsid proteins and change the cell-specificity of Ad transduction. Bispecific adapters were originally developed as a means to block CAR-mediated transduction and retarget Ad to unique cell surface receptors [183-185]. One bispecific adapter that has shown promise for mediating the transduction of normally refractory DCs consists of the soluble domain of CAR fused to the ectodomain of CD40L [186-188]. This binding of CD40 simultaneously leads to Ad transduction and activation of DCs, inducing potent antigen-specific immune responses. *In vitro*, efficient DC transduction was observed in the presence of Ad5 NAbs collected from human patients [189]. This report also indicated the induction of a Th1-skewed antigen-specific immune response following adapter-mediated vector delivery.

These studies demonstrate that significant progress has been made toward modifying Ad vectors to successfully evade Ad PEI and subsequent toxicity, but also highlight the need for further analysis to ensure adequate therapeutic immune responses are generated. Interestingly, the route of vector administration may have a significant impact on the immune response generated to Ad vectors, including polymer-coated vectors.

Routes of administration

Ads successfully infect humans via mucosal routes, so it stands to reason that immunizing via this natural route of transmission allows some circumvention of anti-Ad5 immunity. Indeed, reports have shown that mucosal administration of Ad vectors does not promote the development of anti-Ad immunity but allows the induction of transgene product-specific immune responses [190]. This may be due at least in part to lower levels of anti-Ad5 NAbs

localized in human mucosa compared with in the systemic circulation [191]. Importantly, both systemic and mucosal immunity against Ad-encoded transgene products can be triggered by mucosal vaccination [192-194], and this can provide protection from pathogen infection when administered prophylactically, even in the presence of Ad PEI [190,195-199].

Several laboratories have continued to investigate the benefits that may result from administration of Ad5 vectors by various routes, and whether this can be combined with biochemical modifications to further reduce anti-Ad5 immunity and toxicity. As anticipated, intranasal delivery of unmodified Ad5 vectors in naive mice leads to transgene expression predominantly in the nose and lungs [176,189]. This localized expression should minimize exposure to humoral and cellular anti-Ad5 reactivity and minimize the chances of liver toxicity. However, when administered intranasally, modification of Ad5 with PEG polymers of at least 35 kDa appears necessary to induce transgene expression and antigen-specific antibody levels that are similar to those induced by unmodified Ad5 [176]. Attachment of additional glucose or galactose ligands to 35-kDa PEG moieties on Ad5 further increased transgene expression following intranasal administration, but not intramuscular injection. Sugar ligands on PEGylated vectors may enhance vector stability or the ability to target carbohydrate-specific receptors on antigen-presenting cells (APCs). Interestingly, following intranasal administration in Ad5 naive mice, the lowest levels of anti-Ad5 NAbs were observed in mice vaccinated with 5-kDa PEG modified vectors, while vectors modified with larger PEG polymers induced levels of NAbs similar to unmodified Ad5. It is not clear whether Ad5 PEI would alter the selective inhibition of Ad-humoral responses by intranasal administration of 5-kDa PEG-modified vectors, as this was not specifically evaluated.

The effect of administration route, as well as the impact of vector-specific PEI, was examined with respect to generating antigen-specific T-cell responses against two different transgene products [176]. In naive mice, intramuscular administration of vectors with or without PEG modification (5 or 35 kDa) produced the same level of green fluorescent protein (GFP)-specific T cells. However, intranasal administration produced only half the numbers of GFP-specific T cells, but within this group, 5-kDa PEG-modified vectors significantly increased this response. Interestingly, PEG modification produced different effects on T-cell responses made against the vector-delivered Gag antigen. While intramuscular administration produced higher responses as compared with those generated by intranasal administration, PEGylation (5 and 35 kDa) inhibited the magnitude of the T-cell response. In this case, reduced transduction efficiency by PEGylation may have affected the outcome. In contrast to GFP-specific responses generated by intranasal administration, Gag-specific T-cell responses were not increased by 5-kDa PEGylated vectors. These differences may reflect differences in the time between administration of vectors and the measurement of T-cell responses (7 weeks for GFP; 2 weeks for Gag). Alternatively, the immunogenicity of each encoded antigen may differ and accordingly influence the T-cell response (e.g., GFP is known to be a weak antigen, relative to Gag, with respect to generating a cellular response). In mice with Ad5 PEI, only 5-kDa PEGylated vectors induced Gag-specific T-cell responses similar to those in Ad5-naive mice following intramuscular administration. Also of interest, 5-kDa PEGylated vector-specific PEI only partially diminished the capacity to vaccinate with homologously modified vectors, but did not impact unmodified or 35-kDa PEGylated vaccines. The sugar-modified PEGylated vectors would be of interest to investigate in this scenario as well, especially in light of the enhanced transgene expression observed following administration of these vectors. Thus, the ability to shield against Ad5 PEI through modification with high-molecular-weight PEG polymers may depend on the route of vector administration and the encoded antigen, which can influence the magnitude and quality of the resultant cellular or humoral response.

Using a mouse model of Ebola, Croyle *et al.* investigated the differences in the immune response and survival against an infectious pathogen challenge following immunization by systemic (intramuscular) or mucosal (intranasal, per oral) administration of Ad5 vectors encoding the Ebola Zaire glycoprotein antigen [191]. In mice with Ad5 PEI, transgene product-specific antibodies were only produced when vectors were administered intranasally. In naive mice, intramuscular injection of vector resulted in higher antigen-specific T-cell responses than intranasal or per oral routes of administration. However, Ad5 PEI dramatically reduced T-cell responses generated after intramuscular injection of vectors, but not after intranasal or per oral vector administration. These effects of intranasal administration on T-cell responses induced by unmodified Ad vectors are similar to those observed by Weaver and Barry discussed previously. In the most critical test of vector efficacy, only intranasal administration fully protected mice with Ad5 PEI from a lethal challenge with mouse-adapted Ebola [191].

Hypothesizing that PEGylation may afford additional vector protection against Ad5 PEI, this group also investigated whether PEGylated Ad5 administered orally could improve immune responses and survival after Ebola challenge. Although PEGylation facilitated the production of antigen-specific antibodies in mice with Ad5 PEI, few T cells were detected, and vaccinated mice did not survive Ebola challenge [191]. In light of the studies reviewed in the previous sections, it would be of interest to investigate whether modifying Ad vectors with various sizes of PEG polymers provides additional benefits to orally administered vectors.

These studies clearly point out the potential of mucosal vector delivery, and intranasal administration in particular, for evading Ad PEI. However, concerns were raised about intranasal administration following early studies reporting the detection of transgene product expression in the olfactory bulb [200,201]. Supporting these reports, transgene product expression in mouse brain following intranasal administration of Ad5 vectors was again observed by Huang *et al.* [189]. This transduction appears to be CAR-mediated, since modification with retargeting adapter proteins significantly decreases transgene expression [189]. However, Damjanovic *et al.* found little or no evidence of Ad vector distribution in the brain following intranasal administration of a threefold lower vector dose in the same species [202]. Together, these reports indicate that intranasal administration of Ad vectors may evade Ad PEI and induce powerful immunity to encoded antigens, but vector dose may be a critical consideration.

Cell vehicles

One popular method for Ad5-mediated immunotherapy involves *ex vivo* transduction of DCs with Ad5 vectors. Following cytokine-induced maturation in culture, transduced DCs are re-administered as a vaccine. When evaluated in clinical trials with cancer patients, this method leads to antigen-specific immune responses in approximately 50% of patients, but cancer regression is only achieved in approximately 20% of patients. Several hypotheses have been put forward as explanations, but the effects of Ad PEI were only recently examined. In mice vaccinated with DCs transduced with an Ad5 vector encoding a hepatitis C protein, Ad5 PEI had no effect on the resulting antihepatitis C immune responses [203]. In addition, no anti-Ad5 NAbs are generated by this method. Thus, vaccinating with cells transduced with Ad5 vectors *ex vivo* may provide an effective means for avoiding Ad PEI. However, harvesting and *ex vivo* manipulation of DCs has technical as well as other biologically related issues that need to be addressed before vaccination with transduced cells can become standard [204].

Expert commentary

As previously mentioned, Ad vectors have been utilized for a wide range of vaccine approaches. However, the recent findings of the STEP HIV vaccine clinical trial failed to reproduce promising findings observed in animal model systems [90]. In this regard, this vaccine, which used an Ad5-based vector, failed to protect Ad5-seronegative individuals against infection and was associated with enhanced infection in vaccine recipients with Ad PEI. However, the lack of protection was not altogether surprising, given the previous results in macaques demonstrating that a similar prime–boost strategy with Ad5 vectors encoding SIV antigens did not control viral replication [89,205], and these results have led to a variety of speculative explanations [74,87-90]. In particular, recent studies have pointed to the critical role of cross-reactive T cells in limiting the efficacy of vaccines derived from both human and nonhuman tropic Ads [74,133]. However, most studies in animal models have, to date, focused on evading Ad PEI in the context of NAbs. Overall, these findings emphasize the need for strategies to reduce vector immunogenicity with respect to evading recognition by pre-immune sera and T cells in individuals with Ad PEI, as well as avoiding the induction of new Ad-specific responses in vaccine recipients without PEI. These two accomplishments will be necessary to preserve Ad-based vectors as a premier vector for vaccine development.

Future evaluation should also focus on the effects on DC transduction and immunogenicity. For example, FcR-mediated uptake of Ad vectors by DCs may lead to enhanced transduction and activation of innate and adaptive responses, but polymer coating may impair this uptake and adjuvant activity. Vectors derived from alternative species-tropic Ads have unique DC-specific effects. Some do not induce effective DC activation [126] and behave in a manner similar to human subgroup C Ads (which includes Ad2, -5 and -34), while the nonhuman primate Ad subgroups B (including Ad29, -32 and -46) and E (including Ad4, -24, -36 and -39) strongly stimulate pDCs to produce high levels of IFN-α and other inflammatory cytokines [74]. Such immediate early responses by pDCs may profoundly contribute to the susceptibility thresholds each patient may have for vector toxicity. Thus, each vector subtype has unique immune-stimulatory properties that will need to be considered when designing Ad-based therapeutics and vaccine strategies.

Another potential concern regarding Ad-based vaccines has been highlighted recently, and involves the capacity of Ads to integrate into the cellular genome. It has long been thought that Ad genomic integration occurs at an extremely inefficient rate, thereby providing a safety advantage of this virus for patient vaccination. However, recent reports indicate that Ads can randomly integrate into the genome of some cell cultures *in vitro* at a much higher rate than originally believed (up to 5%), and this may be related to the rate of cell division [206,207]. Thus, in light of the potential side effects of random integration, such as cell transformation (as observed with retroviruses [208]), Ad-based vaccines may require more careful monitoring in this regard than previously expected. On the other hand, studies in mice from Ertl's laboratory suggest that the persistence of transcriptionally active Ad transgenes may prove to be an advantageous property. Although the exact mechanism underlying persistence following Ad vector vaccination was not determined, it was shown to provide sufficiently low levels of transgene product expression to act as a source of homeostatic signals for maintaining a pool of long-term antigen-specific effector memory $CD8^+$ T cells [209]. These effector memory cells could mount a fast lytic response against antigen-bearing cells, potentially providing enhanced vaccine protection.

In addition to shielding and retargeting vectors, improved animal models are needed to study Ad PEI mechanisms and provide more relevant preclinical information. As observed by Croyle *et al.*, NAbs induced in mouse models via intramuscular injection of Ad may not

adequately represent levels observed in human mucosal regions following natural exposure through inhalation [191]. The discovery that CAR is expressed by human and rat erythrocytes, but not by nonhuman primate, mouse, rabbit and dog erythrocytes, is yet another example of the crucial differences between humans and animals that must be addressed [179,210]. Indeed, even immune responses in nonhuman primates must be evaluated further before preclinical results can be properly interpreted with regard to human clinical translation [74]. Long-term efficacy studies to examine the duration of protection afforded by vaccination must also be undertaken. As the routes of vector administration obviously affect therapeutic outcome too, methods for administering therapeutic vectors mucosally, or by other less well-explored routes such as topically, may provide promising new avenues to explore. Such topical administration would be less susceptible to neutralization by humoral or cellular responses to Ad, as these sites are insulated from systemic exposure.

Five-year view

The past few years have brought exciting technical advances, along with critically important information that elucidated some of the immune mechanisms responsible for some of the toxicities and poor therapeutic outcomes in response to Ad vector-based therapeutics in the presence of Ad PEI. These studies have revealed the complexity of factors influencing the efficacy of Ad-based vaccines, and emphasize the stringent conditions in which potential Ad-based therapies must be evaluated. Thus, research in the next few years will undoubtedly focus on two areas:

- Fully characterizing the components of PEI (Ad-opsonizing and NAbs, memory T cells and latent Ad infections) in affecting the efficacy of Ad vector-based vaccines;
- Continuing to explore new technical advances that promote evasion of humoral and cellular recognition of the Ad vector by PEI, and reducing the induction of specific Ad immunity, while maintaining enhanced immunogenicity toward the delivered transgene vaccine product. In this respect, expanding the utility of gutless Ad vectors may continue to be a promising advance.

It is also becoming evident that many factors must be evaluated to optimize each specific vaccine, and careful consideration must be given to determining the unique prime–boost combination of a chosen vector subgroup or strain, vector modifications, polymer coating, epitope–capsid incorporation, transgene selection, vector dose and administration route that will provide an optimal therapeutic outcome for treating or immunizing against each unique disease of interest in patients with Ad PEI. Indeed, routes of vector administration that avoid exposure to immune recognition, such as the transduction of epithelial layer cells in the mucosa or cutaneous tissue, appear to be promising routes to explore to allow realization of the full potential of Ad vector-based vaccines. Finally, with recent understanding of the profound differences in species-specific Ad properties and pathologies among nonhuman primates, mice and humans, it is imperative that animal models that more closely mimic the human biology and pathology of Ad infection are developed and fully defined. Such models will be invaluable for investigating the relevant properties of Ad vectors and the mechanisms that elicit immune responses, or promote immune evasion by vectors. Only then will we be closer to accurately predicting therapeutic outcome of vaccine trials in humans.

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References

Papers of special note have been highlighted as:

- • of interest
- •• of considerable interest

1. Shirakawa T. The current status of adenovirus-based cancer gene therapy. Mol Cell. 2008; 25(4): 462–466.
2. Douglas JT. Adenoviral vectors for gene therapy. Mol Biotechnol. 2007; 36(1):71–80. [PubMed: 17827541]
3. Campos SK, Barry MA. Current advances and future challenges in adenoviral vector biology and targeting. Curr Gene Ther. 2007; 7(3):189–204. [PubMed: 17584037]
4. Goncalves MA, de Vries AA. Adenovirus: from foe to friend. Rev Med Virol. 2006; 16(3):167–186. [PubMed: 16710837]
5. Hartman ZC, Black EP, Amalfitano A. Adenoviral infection induces a multi-faceted innate cellular immune response that is mediated by the Toll-like receptor pathway in A549 cells. Virology. 2007; 358(2):357–372. [PubMed: 17027060]
6. Bai M, Harfe B, Freimuth P. Mutations that alter an Arg–Gly–Asp (RGD) sequence in the adenovirus type 2 penton base protein abolish its cell-rounding activity and delay virus reproduction in flat cells. J Virol. 1993; 67(9):5198–5205. [PubMed: 8350395]
7. Bergelson JM, Cunningham JA, Droguett G, et al. Isolation of a common receptor for Coxsackie B viruses and adenoviruses 2 and 5. Science. 1997; 275(5304):1320–1323. [PubMed: 9036860]
8. Xia D, Henry LJ, Gerard RD, Deisenhofer J. Crystal structure of the receptor-binding domain of adenovirus type 5 fiber protein at 1.7 Å resolution. Structure. 1994; 2(12):1259–1270. [PubMed: 7704534]
9. Wickham TJ, Mathias P, Cheresh DA, Nemerow GR. Integrins α v β 3 and α v β 5 promote adenovirus internalization but not virus attachment. Cell. 1993; 73(2):309–319. [PubMed: 8477447]
10. Wiethoff CM, Wodrich H, Gerace L, Nemerow GR. Adenovirus protein VI mediates membrane disruption following capsid disassembly. J Virol. 2005; 79(4):1992–2000. [PubMed: 15681401]
11. Greber UF, Willetts M, Webster P, Helenius A. Stepwise dismantling of adenovirus 2 during entry into cells. Cell. 1993; 75(3):477–486. [PubMed: 8221887]
12. Saphire AC, Guan T, Schirmer EC, Nemerow GR, Gerace L. Nuclear import of adenovirus DNA *in vitro* involves the nuclear protein import pathway and hsc70. J Biol Chem. 2000; 275(6):4298–4304. [PubMed: 10660598]
13. Trotman LC, Mosberger N, Fornerod M, Stidwill RP, Greber UF. Import of adenovirus DNA involves the nuclear pore complex receptor CAN/Nup214 and histone H1. Nat Cell Biol. 2001; 3(12):1092–1100. [PubMed: 11781571]
14. Greber UF, Suomalainen M, Stidwill RP, et al. The role of the nuclear pore complex in adenovirus DNA entry. EMBO J. 1997; 16(19):5998–6007. [PubMed: 9312057]
15. Schnell MA, Zhang Y, Tazelaar J, et al. Activation of innate immunity in nonhuman primates following intraportal administration of adenoviral vectors. Mol Ther. 2001; 3(5 Pt 1):708–722. [PubMed: 11356076]
16. Raper SE, Chirmule N, Lee FS, et al. Fatal systemic inflammatory response syndrome in a ornithine transcarbamylase deficient patient following adenoviral gene transfer. Mol Genet Metab. 2003; 80(1–2):148–158. [PubMed: 14567964]
17. Muruve DA, Barnes MJ, Stillman IE, Libermann TA. Adenoviral gene therapy leads to rapid induction of multiple chemokines and acute neutrophil-dependent hepatic injury *in vivo*. Hum Gene Ther. 1999; 10(6):965–976. [PubMed: 10223730]
18. Lieber A, He CY, Meuse L, et al. The role of Kupffer cell activation and viral gene expression in early liver toxicity after infusion of recombinant adenovirus vectors. J Virol. 1997; 71(11):8798–8807. [PubMed: 9343240]

19. Zhang Y, Chirmule N, Gao GP, et al. Acute cytokine response to systemic adenoviral vectors in mice is mediated by dendritic cells and macrophages. Mol Ther. 2001; 3(5 Pt 1):697–707. [PubMed: 11356075]
20. Cichon G, Boeckh-Herwig S, Schmidt HH, et al. Complement activation by recombinant adenoviruses. Gene Ther. 2001; 8(23):1794–1800. [PubMed: 11803399]
21. Jiang H, Wang Z, Serra D, Frank MM, Amalfitano A. Recombinant adenovirus vectors activate the alternative complement pathway, leading to the binding of human complement protein C3 independent of anti-ad antibodies. Mol Ther. 2004; 10(6):1140–1142. [PubMed: 15564145]
22. Kiang A, Hartman ZC, Everett RS, et al. Multiple innate inflammatory responses induced after systemic adenovirus vector delivery depend on a functional complement system. Mol Ther. 2006; 14(4):588–598. [PubMed: 16733096]
23. Mistchenko AS, Lenzi HL, Thompson FM, et al. Participation of immune complexes in adenovirus infection. Acta Paediatr. 1992; 81(12):983–988. [PubMed: 1290863]
24. Machemer T, Engler H, Tsai V, et al. Characterization of hemodynamic events following intravascular infusion of recombinant adenovirus reveals possible solutions for mitigating cardiovascular responses. Mol Ther. 2005; 12(2):254–263. [PubMed: 16043097]
25. Tao N, Gao GP, Parr M, et al. Sequestration of adenoviral vector by Kupffer cells leads to a nonlinear dose response of transduction in liver. Mol Ther. 2001; 3(1):28–35. [PubMed: 11162308]
26. Schiedner G, Bloch W, Hertel S, et al. A hemodynamic response to intravenous adenovirus vector particles is caused by systemic Kupffer cell-mediated activation of endothelial cells. Hum Gene Ther. 2003; 14(17):1631–1641. [PubMed: 14633405]
27. Bruder JT, Kovesdi I. Adenovirus infection stimulates the Raf/MAPK signaling pathway and induces interleukin-8 expression. J Virol. 1997; 71(1):398–404. [PubMed: 8985363]
28. Suomalainen M, Nakano MY, Boucke K, Keller S, Greber UF. Adenovirus-activated PKA and p38/MAPK pathways boost microtubule-mediated nuclear targeting of virus. EMBO J. 2001; 20(6):1310–1319. [PubMed: 11250897]
29. Tibbles LA, Spurrell JC, Bowen GP, et al. Activation of p38 and ERK signaling during adenovirus vector cell entry lead to expression of the C–X–C chemokine IP-10. J Virol. 2002; 76(4):1559–1568. [PubMed: 11799150]
30. Liu Q, White LR, Clark SA, et al. Akt/protein kinase B activation by adenovirus vectors contributes to NFκB-dependent CXCL10 expression. J Virol. 2005; 79(23):14507–14515. [PubMed: 16282450]
31. Parker AL, Nicklin SA, Baker AH. Interactions of adenovirus vectors with blood: implications for intravascular gene therapy applications. Curr Opin Mol Ther. 2008; 10(5):439–448. [PubMed: 18830919]
32. Sakurai H, Kawabata K, Sakurai F, Nakagawa S, Mizuguchi H. Innate immune response induced by gene delivery vectors. Int J Pharm. 2008; 354(1–2):9–15. [PubMed: 17640834]
33. Hartman ZC, Appledorn DM, Amalfitano A. Adenovirus vector induced innate immune responses: impact upon efficacy and toxicity in gene therapy and vaccine applications. Virus Res. 2008; 132(1–2):1–14. [PubMed: 18036698]
34. Wu TL, Ertl HC. Immune barriers to successful gene therapy. Trends Mol Med. 2009; 15(1):32–39. [PubMed: 19101205]
35. Zinn KR, Szalai AJ, Stargel A, Krasnykh V, Chaudhuri TR. Bioluminescence imaging reveals a significant role for complement in liver transduction following intravenous delivery of adenovirus. Gene Ther. 2004; 11(19):1482–1486. [PubMed: 15295616]
36. Appledorn DM, McBride A, Seregin S, et al. Complex interactions with several arms of the complement system dictate innate and humoral immunity to adenoviral vectors. Gene Ther. 2008; 15(24):1606–1617. [PubMed: 18615115]
37. Xu Z, Tian J, Smith JS, Byrnes AP. Clearance of adenovirus by Kupffer cells is mediated by scavenger receptors, natural antibodies, and complement. J Virol. 2008; 82(23):11705–11713. [PubMed: 18815305]

38. Cichon G, Schmidt HH, Benhidjeb T, et al. Intravenous administration of recombinant adenoviruses causes thrombocytopenia, anemia and erythroblastosis in rabbits. J Gene Med. 1999; 1(5):360–371. [PubMed: 10738553]
39. Stone D, Liu Y, Shayakhmetov D, et al. Adenovirus–platelet interaction in blood causes virus sequestration to the reticuloendothelial system of the liver. J Virol. 2007; 81(9):4866–4871. [PubMed: 17301138]
40. Othman M, Labelle A, Mazzetti I, Elbatarny HS, Lillicrap D. Adenovirus-induced thrombocytopenia: the role of von Willebrand factor and P-selectin in mediating accelerated platelet clearance. Blood. 2007; 109(7):2832–2839. [PubMed: 17148587]
41. Cotter MJ, Zaiss AK, Muruve DA. Neutrophils interact with adenovirus vectors via Fc receptors and complement receptor 1. J Virol. 2005; 79(23):14622–14631. [PubMed: 16282462]
42. Shayakhmetov DM, Gaggar A, Ni S, Li ZY, Lieber A. Adenovirus binding to blood factors results in liver cell infection and hepatotoxicity. J Virol. 2005; 79(12):7478–7491. [PubMed: 15919903]
43. Parker AL, Waddington SN, Nicol CG, et al. Multiple vitamin K-dependent coagulation zymogens promote adenovirus-mediated gene delivery to hepatocytes. Blood. 2006; 108(8):2554–2561. [PubMed: 16788098]
44. Smith JG, Cassany A, Gerace L, Ralston R, Nemerow GR. Neutralizing antibody blocks adenovirus infection by arresting microtubule-dependent cytoplasmic transport. J Virol. 2008; 82(13):6492–6500. [PubMed: 18448546]
45. Haisma HJ, Kamps JA, Kamps GK, et al. Polyinosinic acid enhances delivery of adenovirus vectors *in vivo* by preventing sequestration in liver macrophages. J Gen Virol. 2008; 89(Pt 5): 1097–1105. [PubMed: 18420786]
46. Smith JG, Nemerow GR. Mechanism of adenovirus neutralization by human α-defensins. Cell Host Microbe. 2008; 3(1):11–19. [PubMed: 18191790]
47. Ginsberg HS. The ups and downs of adenovirus vectors. Bull NY Acad Med. 1996; 73(1):53–58.
48. Chirmule N, Propert K, Magosin S, et al. Immune responses to adenovirus and adeno-associated virus in humans. Gene Ther. 1999; 6(9):1574–1583. [PubMed: 10490767]
49. Kass-Eisler A, Falck-Pedersen E, Elfenbein DH, et al. The impact of developmental stage, route of administration and the immune system on adenovirus-mediated gene transfer. Gene Ther. 1994; 1(6):395–402. [PubMed: 7584105]
50. Kass-Eisler A, Leinwand L, Gall J, Bloom B, Falck-Pedersen E. Circumventing the immune response to adenovirus-mediated gene therapy. Gene Ther. 1996; 3(2):154–162. [PubMed: 8867863]
51. Wohlfart C. Neutralization of adenoviruses: kinetics, stoichiometry, and mechanisms. J Virol. 1988; 62(7):2321–2328. [PubMed: 3373570]
52. Olive M, Eisenlohr LC, Flomenberg P. Quantitative analysis of adenovirus-specific $CD4^+$ T-cell responses from healthy adults. Viral Immunol. 2001; 14(4):403–413. [PubMed: 11792069]
53. Crystal RG. Transfer of genes to humans: early lessons and obstacles to success. Science. 1995; 270(5235):404–410. [PubMed: 7569994]
54. Yang Y, Wilson JM. Clearance of adenovirus-infected hepatocytes by MHC class I-restricted $CD4^+$ CTLs *in vivo*. J Immunol. 1995; 155(5):2564–2570. [PubMed: 7650386]
55. Sumida SM, Truitt DM, Kishko MG, et al. Neutralizing antibodies and $CD8^+$ T lymphocytes both contribute to immunity to adenovirus serotype 5 vaccine vectors. J Virol. 2004; 78(6):2666–2673. [PubMed: 14990686]
56. McKelvey T, Tang A, Bett AJ, Casimiro DR, Chastain M. T-cell response to adenovirus hexon and DNA-binding protein in mice. Gene Ther. 2004; 11(9):791–796. [PubMed: 14961070]
57. Molinier-Frenkel V, Gahery-Segard H, Mehtali M, et al. Immune response to recombinant adenovirus in humans: capsid components from viral input are targets for vector-specific cytotoxic T lymphocytes. J Virol. 2000; 74(16):7678–7682. [PubMed: 10906225]
58. Albert ML, Sauter B, Bhardwaj N. Dendritic cells acquire antigen from apoptotic cells and induce class I-restricted CTLs. Nature. 1998; 392(6671):86–89. [PubMed: 9510252]
59. Muruve DA, Petrilli V, Zaiss AK, et al. The inflammasome recognizes cytosolic microbial and host DNA and triggers an innate immune response. Nature. 2008; 452(7183):103–107. [PubMed: 18288107]

60. Cerullo V, Seiler MP, Mane V, et al. Toll-like receptor 9 triggers an innate immune response to helper-dependent adenoviral vectors. Mol Ther. 2007; 15(2):378–385. [PubMed: 17235317]
61. Iacobelli-Martinez M, Nemerow GR. Preferential activation of Toll-like receptor nine by CD46-utilizing adenoviruses. J Virol. 2007; 81(3):1305–1312. [PubMed: 17108047]
62. Basner-Tschakarjan E, Gaffal E, O'Keeffe M, et al. Adenovirus efficiently transduces plasmacytoid dendritic cells resulting in TLR9-dependent maturation and IFN-α production. J Gene Med. 2006; 8(11):1300–1306. [PubMed: 16952196]
63. Zhu J, Huang X, Yang Y. Innate immune response to adenoviral vectors is mediated by both Toll-like receptor-dependent and -independent pathways. J Virol. 2007; 81(7):3170–3180. [PubMed: 17229689]
64. Appledorn DM, Patial S, McBride A, et al. Adenovirus vector-induced innate inflammatory mediators, MAPK signaling, as well as adaptive immune responses are dependent upon both TLR2 and TLR9 *in vivo*. J Immunol. 2008; 181(3):2134–2144. [PubMed: 18641352]
65. Jankovic D, Liu Z, Gause WC. Th1- and Th2-cell commitment during infectious disease: asymmetry in divergent pathways. Trends Immunol. 2001; 22(8):450–457. [PubMed: 11473835]
66. Vujanovic L, Whiteside TL, Potter DM, et al. Regulation of antigen presentation machinery in human dendritic cells by recombinant adenovirus. Cancer Immunol Immunother. 2009; 58(1):121–133. [PubMed: 18488218]
67. Evdokimova VN, Liu Y, Potter DM, Butterfield LH. AFP-specific $CD4^+$ helper T-cell responses in healthy donors and HCC patients. J Immunother. 2007; 30(4):425–437. [PubMed: 17457217]
68. Schumacher L, Ribas A, Dissette VB, et al. Human dendritic cell maturation by adenovirus transduction enhances tumor antigen-specific T-cell responses. J Immunother. 2004; 27(3):191–200. [PubMed: 15076136]
69. Lundqvist A, Choudhury A, Nagata T, et al. Recombinant adenovirus vector activates and protects human monocyte-derived dendritic cells from apoptosis. Hum Gene Ther. 2002; 13(13):1541–1549. [PubMed: 12228009]
70. Varnavski AN, Schlienger K, Bergelson JM, Gao GP, Wilson JM. Efficient transduction of human monocyte-derived dendritic cells by chimpanzee-derived adenoviral vector. Hum Gene Ther. 2003; 14(6):533–544. [PubMed: 12718764]
71. Korst RJ, Mahtabifard A, Yamada R, Crystal RG. Effect of adenovirus gene transfer vectors on the immunologic functions of mouse dendritic cells. Mol Ther. 2002; 5(3):307–315. [PubMed: 11863421]
72. Dietz AB, Vuk-Pavlovic S. High efficiency adenovirus-mediated gene transfer to human dendritic cells. Blood. 1998; 91(2):392–398. [PubMed: 9427691]
73. Hirschowitz EA, Weaver JD, Hidalgo GE, Doherty DE. Murine dendritic cells infected with adenovirus vectors show signs of activation. Gene Ther. 2000; 7(13):1112–1120. [PubMed: 10918477]
74••. Calcedo R, Vandenberghe LH, Roy S, et al. Host immune responses to chronic adenovirus infections in human and nonhuman primates. J Virol. 2009; 83(6):2623–2631. Reveals profound differences in adenovirus (Ad) pathogenesis and immune response mechanisms that are elaborated in nonhuman primates, as compared with humans. Differences in nonhuman primate-specific Ad subgroups are also characterized with respect to their capacity to stimulate high levels of IFN-γ from human plasmacytoid dendritic cells (pDCs). [PubMed: 19116257]
75. Smith CA, Woodruff LS, Rooney C, Kitchingman GR. Extensive cross-reactivity of adenovirus-specific cytotoxic T cells. Hum Gene Ther. 1998; 9(10):1419–1427. [PubMed: 9681413]
76. Heemskerk B, van Vreeswijk T, Veltrop-Duits LA, et al. Adenovirus-specific $CD4^+$ T cell clones recognizing endogenous antigen inhibit viral replication *in vitro* through cognate interaction. J Immunol. 2006; 177(12):8851–8859. [PubMed: 17142788]
77. Haveman LM, Bierings M, Legger E, et al. Novel pan-DR-binding T cell epitopes of adenovirus induce pro-inflammatory cytokines and chemokines in healthy donors. Int Immunol. 2006; 18(11): 1521–1529. [PubMed: 16954167]
78. Sparer TE, Wynn SG, Clark DJ, et al. Generation of cytotoxic T lymphocytes against immunorecessive epitopes after multiple immunizations with adenovirus vectors is dependent on haplotype. J Virol. 1997; 71(3):2277–2284. [PubMed: 9032363]

79. Jooss K, Ertl HC, Wilson JM. Cytotoxic T-lymphocyte target proteins and their major histocompatibility complex class I restriction in response to adenovirus vectors delivered to mouse liver. J Virol. 1998; 72(4):2945–2954. [PubMed: 9525615]
80. Leen AM, Sili U, Vanin EF, et al. Conserved CTL epitopes on the adenovirus hexon protein expand subgroup cross-reactive and subgroup-specific $CD8^+$ T cells. Blood. 2004; 104(8):2432–2440. [PubMed: 15265797]
81. Olive M, Eisenlohr L, Flomenberg N, Hsu S, Flomenberg P. The adenovirus capsid protein hexon contains a highly conserved human $CD4^+$ T-cell epitope. Hum Gene Ther. 2002; 13(10):1167–1178. [PubMed: 12133270]
82. Tang J, Olive M, Pulmanausahakul R, et al. Human $CD8^+$ cytotoxic T cell responses to adenovirus capsid proteins. Virology. 2006; 350(2):312–322. [PubMed: 16499941]
83. Joshi A, Tang J, Kuzma M, et al. Adenovirus DNA polymerase is recognized by human $CD8^+$ T cells. J Gen Virol. 2009; 90(Pt 1):84–94. [PubMed: 19088277]
84. Flomenberg P, Piaskowski V, Truitt RL, Casper JT. Human adenovirus-specific $CD8^+$ T-cell responses are not inhibited by E3–19K in the presence of γ interferon. J Virol. 1996; 70(9):6314–6322. [PubMed: 8709259]
85. Burgert HG, Kvist S. An adenovirus type 2 glycoprotein blocks cell surface expression of human histocompatibility class I antigens. Cell. 1985; 41(3):987–997. [PubMed: 3924414]
86. Bennett EM, Bennink JR, Yewdell JW, Brodsky FM. Cutting edge: adenovirus E19 has two mechanisms for affecting class I MHC expression. J Immunol. 1999; 162(9):5049–5052. [PubMed: 10227971]
87. Perreau M, Pantaleo G, Kremer EJ. Activation of a dendritic cell-T cell axis by Ad5 immune complexes creates an improved environment for replication of HIV in T cells. J Exp Med. 2008; 205(12):2717–2725. [PubMed: 18981239]
88. Uberla K. HIV vaccine development in the aftermath of the STEP study: re-focus on occult HIV infection? PLoS Pathog. 2008; 4(8):e1000114. [PubMed: 18769723]
89. Watkins DI, Burton DR, Kallas EG, Moore JP, Koff WC. Nonhuman primate models and the failure of the Merck HIV-1 vaccine in humans. Nat Med. 2008; 14(6):617–621. [PubMed: 18535579]
90. Sekaly RP. The failed HIV Merck vaccine study: a step back or a launching point for future vaccine development? J Exp Med. 2008; 205(1):7–12. [PubMed: 18195078]
91. Gahery-Segard H, Farace F, Godfrin D, et al. Immune response to recombinant capsid proteins of adenovirus in humans: antifiber and anti-penton base antibodies have a synergistic effect on neutralizing activity. J Virol. 1998; 72(3):2388–2397. [PubMed: 9499099]
92. Molinier-Frenkel V, Lengagne R, Gaden F, et al. Adenovirus hexon protein is a potent adjuvant for activation of a cellular immune response. J Virol. 2002; 76(1):127–135. [PubMed: 11739678]
93. Wohlfart CE, Svensson UK, Everitt E. Interaction between HeLa cells and adenovirus type 2 virions neutralized by different antisera. J Virol. 1985; 56(3):896–903. [PubMed: 4068145]
94. Toogood CI, Crompton J, Hay RT. Antipeptide antisera define neutralizing epitopes on the adenovirus hexon. J Gen Virol. 1992; 73(Pt 6):1429–1435. [PubMed: 1376769]
95. Varga MJ, Bergman T, Everitt E. Antibodies with specificities against a dispase-produced 15-kilodalton hexon fragment neutralize adenovirus type 2 infectivity. J Virol. 1990; 64(9):4217–4225. [PubMed: 1696636]
96. Varghese R, Mikyas Y, Stewart PL, Ralston R. Postentry neutralization of adenovirus type 5 by an antihexon antibody. J Virol. 2004; 78(22):12320–12332. [PubMed: 15507619]
97. Sumida SM, Truitt DM, Lemckert AA, et al. Neutralizing antibodies to adenovirus serotype 5 vaccine vectors are directed primarily against the adenovirus hexon protein. J Immunol. 2005; 174(11):7179–7185. [PubMed: 15905562]
98. Bangari DS, Mittal SK. Current strategies and future directions for eluding adenoviral vector immunity. Curr Gene Ther. 2006; 6(2):215–226. [PubMed: 16611043]
99. Mane VP, Toietta G, McCormack WM, et al. Modulation of TNFα, a determinant of acute toxicity associated with systemic delivery of first-generation and helper-dependent adenoviral vectors. Gene Ther. 2006; 13(17):1272–1280. [PubMed: 16708078]

100. Price AR, Limberis MP, Wilson JM, Diamond SL. Pulmonary delivery of adenovirus vector formulated with dexamethasone-spermine facilitates homologous vector re-administration. Gene Ther. 2007; 14(22):1594–1604. [PubMed: 17898795]

101. Seregin SS, Appledorn DM, McBride AJ, et al. Transient pretreatment with glucocorticoid ablates innate toxicity of systemically delivered adenoviral vectors without reducing efficacy. Mol Ther. 2009; 17(4):685–696. [PubMed: 19174760]

102. Ishimoto S, Kawamoto K, Stover T, et al. A glucocorticoid reduces adverse effects of adenovirus vectors in the cochlea. Audiol Neurootol. 2003; 8(2):70–79. [PubMed: 12634455]

103. Ramshaw IA, Ramsay AJ. The prime–boost strategy: exciting prospects for improved vaccination. Immunol Today. 2000; 21(4):163–165. [PubMed: 10740236]

104. Mastrangeli A, Harvey BG, Yao J, et al. 'Sero-switch' adenovirus-mediated *in vivo* gene transfer: circumvention of anti-adenovirus humoral immune defenses against repeat adenovirus vector administration by changing the adenovirus serotype. Hum Gene Ther. 1996; 7(1):79–87. [PubMed: 8825871]

105. Moffatt S, Hays J, HogenEsch H, Mittal SK. Circumvention of vector-specific neutralizing antibody response by alternating use of human and non-human adenoviruses: implications in gene therapy. Virology. 2000; 272(1):159–167. [PubMed: 10873758]

106. Farina SF, Gao GP, Xiang ZQ, et al. Replication-defective vector based on a chimpanzee adenovirus. J Virol. 2001; 75(23):11603–11613. [PubMed: 11689642]

107. Lemckert AA, Grimbergen J, Smits S, et al. Generation of a novel replication-incompetent adenoviral vector derived from human adenovirus type 49: manufacture on PER.C6 cells, tropism and immunogenicity. J Gen Virol. 2006; 87(Pt 10):2891–2899. [PubMed: 16963747]

108. Shayakhmetov DM, Lieber A. Dependence of adenovirus infectivity on length of the fiber shaft domain. J Virol. 2000; 74(22):10274–10286. [PubMed: 11044071]

109. Kidd AH, Chroboczek J, Cusack S, Ruigrok RW. Adenovirus type 40 virions contain two distinct fibers. Virology. 1993; 192(1):73–84. [PubMed: 8517033]

110. Yeh HY, Pieniazek N, Pieniazek D, Gelderblom H, Luftig RB. Human adenovirus type 41 contains two fibers. Virus Res. 1994; 33(2):179–198. [PubMed: 7975882]

111. Nakamura T, Sato K, Hamada H. Reduction of natural adenovirus tropism to the liver by both ablation of fiber-Coxsackievirus and adenovirus receptor interaction and use of replaceable short fiber. J Virol. 2003; 77(4):2512–2521. [PubMed: 12551989]

112. Shayakhmetov DM, Li ZY, Ni S, Lieber A. Analysis of adenovirus sequestration in the liver, transduction of hepatic cells, and innate toxicity after injection of fiber-modified vectors. J Virol. 2004; 78(10):5368–5381. [PubMed: 15113916]

113. Schoggins JW, Nociari M, Philpott N, Falck-Pedersen E. Influence of fiber detargeting on adenovirus-mediated innate and adaptive immune activation. J Virol. 2005; 79(18):11627–11637. [PubMed: 16140740]

114. Schoggins JW, Falck-Pedersen E. Fiber and penton base capsid modifications yield diminished adenovirus type 5 transduction and proinflammatory gene expression with retention of antigen-specific humoral immunity. J Virol. 2006; 80(21):10634–10644. [PubMed: 16943295]

115. Roy S, Gao G, Lu Y, et al. Characterization of a family of chimpanzee adenoviruses and development of molecular clones for gene transfer vectors. Hum Gene Ther. 2004; 15(5):519–530. [PubMed: 15144581]

116••. Kobinger GP, Feldmann H, Zhi Y, et al. Chimpanzee adenovirus vaccine protects against Zaire Ebola virus. Virology. 2006; 346(2):394–401. Demonstrates that a single intramuscular injection of AdC7 encoding an Ebola glycoprotein antigen can provide complete, but dose-dependent, protection from lethal Ebola challenge. [PubMed: 16356525]

117. Zhi Y, Figueredo J, Kobinger GP, et al. Efficacy of severe acute respiratory syndrome vaccine based on a nonhuman primate adenovirus in the presence of immunity against human adenovirus. Hum Gene Ther. 2006; 17(5):500–506. [PubMed: 16716107]

118. Fitzgerald JC, Gao GP, Reyes-Sandoval A, et al. A simian replication-defective adenoviral recombinant vaccine to HIV-1 gag. J Immunol. 2003; 170(3):1416–1422. [PubMed: 12538702]

119. Reyes-Sandoval A, Sridhar S, Berthoud T, et al. Single-dose immunogenicity and protective efficacy of simian adenoviral vectors against *Plasmodium berghei*. Eur J Immunol. 2008; 38(3): 732–741. [PubMed: 18266272]

120. Sridhar S, Reyes-Sandoval A, Draper SJ, et al. Single-dose protection against *Plasmodium berghei* by a simian adenovirus vector using a human cytomegalovirus promoter containing intron A. J Virol. 2008; 82(8):3822–3833. [PubMed: 18256155]

121. Xiang Z, Li Y, Cun A, et al. Chimpanzee adenovirus antibodies in humans, sub-Saharan Africa. Emerg Infect Dis. 2006; 12(10):1596–1599. [PubMed: 17176582]

122•. Singh N, Pandey A, Jayashankar L, Mittal SK. Bovine adenoviral vector-based H5N1 influenza vaccine overcomes exceptionally high levels of pre-existing immunity against human adenovirus. Mol Ther. 2008; 16(5):965–971. Investigates homologous and heterologous prime–boost regimens with bovine Ad3 and human Ad5 vectors encoding the H5N1 influenza antigen. In mice with Ad pre-existing immunity (PEI), the Ad3–Ad5 regimen (injected intramuscularly) provided complete protection from lethal influenza challenge, while an Ad5–Ad5 regimen did not. [PubMed: 18301400]

123. Perreau M, Mennechet F, Serratrice N, et al. Contrasting effects of human, canine, and hybrid adenovirus vectors on the phenotypical and functional maturation of human dendritic cells: implications for clinical efficacy. J Virol. 2007; 81(7):3272–3284. [PubMed: 17229706]

124. Sharma A, Bangari DS, Tandon M, et al. Comparative analysis of vector biodistribution, persistence and gene expression following intravenous delivery of bovine, porcine and human adenoviral vectors in a mouse model. Virology. 2009; 386(1):44–54. [PubMed: 19211122]

125. Logunov DY, Zubkova OV, Karyagina-Zhulina AS, et al. Identification of HI-like loop in CELO adenovirus fiber for incorporation of receptor binding motifs. J Virol. 2007; 81(18):9641–9652. [PubMed: 17596306]

126. Perreau M, Kremer EJ. The conundrum between immunological memory to adenovirus and their use as vectors in clinical gene therapy. Mol Biotechnol. 2006; 34(2):247–256. [PubMed: 17172670]

127. Liu J, Ewald BA, Lynch DM, et al. Magnitude and phenotype of cellular immune responses elicited by recombinant adenovirus vectors and heterologous prime–boost regimens in rhesus monkeys. J Virol. 2008; 82(10):4844–4852. [PubMed: 18337575]

128. Wille-Reece U, Flynn BJ, Lore K, et al. Toll-like receptor agonists influence the magnitude and quality of memory T cell responses after prime–boost immunization in nonhuman primates. J Exp Med. 2006; 203(5):1249–1258. [PubMed: 16636134]

129. Wille-Reece U, Flynn BJ, Lore K, et al. HIV Gag protein conjugated to a Toll-like receptor 7/8 agonist improves the magnitude and quality of Th1 and $CD8^+$ T cell responses in nonhuman primates. Proc Natl Acad Sci USA. 2005; 102(42):15190–15194. [PubMed: 16219698]

130. Mattapallil JJ, Douek DC, Buckler-White A, et al. Vaccination preserves CD4 memory T cells during acute simian immunodeficiency virus challenge. J Exp Med. 2006; 203(6):1533–1541. [PubMed: 16735692]

131. Letvin NL, Mascola JR, Sun Y, et al. Preserved $CD4^+$ central memory T cells and survival in vaccinated SIV-challenged monkeys. Science. 2006; 312(5779):1530–1533. [PubMed: 16763152]

132. Liu J, O'Brien KL, Lynch DM, et al. Immune control of an SIV challenge by a T-cell-based vaccine in rhesus monkeys. Nature. 2009; 457(7225):87–91. [PubMed: 18997770]

133•. Schirmbeck R, Reimann J, Kochanek S, Kreppel F. The immunogenicity of adenovirus vectors limits the multispecificity of CD8 T-cell responses to vector-encoded transgenic antigens. Mol Ther. 2008; 16(9):1609–1616. Provides evidence that, even in naive or B-cell-deficient mice, the Ad-specific T cells generated following vaccination with an antigen-encoding Ad vector can limit the diversity of T cells generated against the antigen. [PubMed: 18612271]

134. Wu H, Han T, Belousova N, et al. Identification of sites in adenovirus hexon for foreign peptide incorporation. J Virol. 2005; 79(6):3382–3390. [PubMed: 15731232]

135. Youil R, Toner TJ, Su Q, et al. Hexon gene switch strategy for the generation of chimeric recombinant adenovirus. Hum Gene Ther. 2002; 13(2):311–320. [PubMed: 11812286]

136. Vigne E, Mahfouz I, Dedieu JF, et al. RGD inclusion in the hexon monomer provides adenovirus type 5-based vectors with a fiber knob-independent pathway for infection. J Virol. 1999; 73(6): 5156–5161. [PubMed: 10233980]

137. Roberts DM, Nanda A, Havenga MJ, et al. Hexon-chimaeric adenovirus serotype 5 vectors circumvent pre-existing anti-vector immunity. Nature. 2006; 441(7090):239–243. [PubMed: 16625206]

138•. Kalyuzhniy O, Di Paolo NC, Silvestry M, et al. Adenovirus serotype 5 hexon is critical for virus infection of hepatocytes *in vivo*. Proc Natl Acad Sci USA. 2008; 105(14):5483–5488. Identifies hexon as the major Ad-binding site for coagulation factor X, and demonstrates hexon mutation can prevent binding and factor X-mediated liver transduction following systemic Ad vector administration in mice. This study further identifies an unsuspected role for Ad hexon in intracellular trafficking of Ad vectors in some cell types, suggesting a need for careful evaluation of hexon-modified Ads. [PubMed: 18391209]

139•. Waddington SN, McVey JH, Bhella D, et al. Adenovirus serotype 5 hexon mediates liver gene transfer. Cell. 2008; 132(3):397–409. Identifies hexon as the major Ad-binding site for coagulation factor X, and demonstrates this binding can be blocked by hypervariable region pseudotyping or with factor X-binding protein toxin, preventing liver transduction following systemic Ad vector administration in mice. [PubMed: 18267072]

140. Crompton J, Toogood CI, Wallis N, Hay RT. Expression of a foreign epitope on the surface of the adenovirus hexon. J Gen Virol. 1994; 75(Pt 1):133–139. [PubMed: 7509367]

141. Einfeld DA, Brough DE, Roelvink PW, Kovesdi I, Wickham TJ. Construction of a pseudoreceptor that mediates transduction by adenoviruses expressing a ligand in fiber or penton base. J Virol. 1999; 73(11):9130–9136. [PubMed: 10516019]

142. Meulenbroek RA, Sargent KL, Lunde J, Jasmin BJ, Parks RJ. Use of adenovirus protein IX (pIX) to display large polypeptides on the virion: generation of fluorescent virus through the incorporation of pIX-GFP. Mol Ther. 2004; 9(4):617–624. [PubMed: 15093192]

143. Dmitriev IP, Kashentseva EA, Curiel DT. Engineering of adenovirus vectors containing heterologous peptide sequences in the C terminus of capsid protein IX. J Virol. 2002; 76(14): 6893–6899. [PubMed: 12072490]

144. Krause A, Joh JH, Hackett NR, et al. Epitopes expressed in different adenovirus capsid proteins induce different levels of epitope-specific immunity. J Virol. 2006; 80(11):5523–5530. [PubMed: 16699033]

145. Vigant F, Descamps D, Jullienne B, et al. Substitution of hexon hypervariable region 5 of adenovirus serotype 5 abrogates blood factor binding and limits gene transfer to liver. Mol Ther. 2008; 16(8):1474–1480. [PubMed: 18560416]

146. Worgall S, Krause A, Rivara M, et al. Protection against *P. aeruginosa* with an adenovirus vector containing an OprF epitope in the capsid. J Clin Invest. 2005; 115(5):1281–1289. [PubMed: 15841217]

147. Matthews QL, Yang P, Wu Q, et al. Optimization of capsid-incorporated antigens for a novel adenovirus vaccine approach. Virol J. 2008; 5:98. [PubMed: 18718011]

148. Brown BD, Shi CX, Rawle FE, et al. Factors influencing therapeutic efficacy and the host immune response to helper-dependent adenoviral gene therapy in hemophilia A mice. J Thromb Haemost. 2004; 2(1):111–118. [PubMed: 14717974]

149. Kim IH, Jozkowicz A, Piedra PA, Oka K, Chan L. Lifetime correction of genetic deficiency in mice with a single injection of helper-dependent adenoviral vector. Proc Natl Acad Sci USA. 2001; 98(23):13282–13287. [PubMed: 11687662]

150. Morral N, O'Neal W, Rice K, et al. Administration of helper-dependent adenoviral vectors and sequential delivery of different vector serotype for long-term liver-directed gene transfer in baboons. Proc Natl Acad Sci USA. 1999; 96(22):12816–12821. [PubMed: 10536005]

151. Kafri T, Morgan D, Krahl T, et al. Cellular immune response to adenoviral vector infected cells does not require *de novo* viral gene expression: implications for gene therapy. Proc Natl Acad Sci USA. 1998; 95(19):11377–11382. [PubMed: 9736744]

152. Roth MD, Cheng Q, Harui A, et al. Helper-dependent adenoviral vectors efficiently express transgenes in human dendritic cells but still stimulate antiviral immune responses. J Immunol. 2002; 169(8):4651–4656. [PubMed: 12370405]
153. Brunetti-Pierri N, Palmer DJ, Beaudet AL, et al. Acute toxicity after high-dose systemic injection of helper-dependent adenoviral vectors into nonhuman primates. Hum Gene Ther. 2004; 15(1): 35–46. [PubMed: 14965376]
154. Muruve DA, Cotter MJ, Zaiss AK, et al. Helper-dependent adenovirus vectors elicit intact innate but attenuated adaptive host immune responses *in vivo*. J Virol. 2004; 78(11):5966–5972. [PubMed: 15140994]
155. Wagner E. Converging paths of viral and non-viral vector engineering. Mol Ther. 2008; 16(1):1–2. [PubMed: 18097464]
156. Kreppel F, Kochanek S. Modification of adenovirus gene transfer vectors with synthetic polymers: a scientific review and technical guide. Mol Ther. 2008; 16(1):16–29. [PubMed: 17912234]
157. O'Riordan CR, Song A. PEGylated adenovirus for targeted gene therapy. Methods Mol Biol. 2008; 434:133–160. [PubMed: 18470643]
158. Eto Y, Yoshioka Y, Mukai Y, Okada N, Nakagawa S. Development of PEGylated adenovirus vector with targeting ligand. Int J Pharm. 2008; 354(1–2):3–8. [PubMed: 17904316]
159. Romanczuk H, Galer CE, Zabner J, et al. Modification of an adenoviral vector with biologically selected peptides: a novel strategy for gene delivery to cells of choice. Hum Gene Ther. 1999; 10(16):2615–2626. [PubMed: 10566889]
160. Croyle MA, Chirmule N, Zhang Y, Wilson JM. 'Stealth' adenoviruses blunt cell-mediated and humoral immune responses against the virus and allow for significant gene expression upon readministration in the lung. J Virol. 2001; 75(10):4792–4801. [PubMed: 11312351]
161. Croyle MA, Yu QC, Wilson JM. Development of a rapid method for the PEGylation of adenoviruses with enhanced transduction and improved stability under harsh storage conditions. Hum Gene Ther. 2000; 11(12):1713–1722. [PubMed: 10954905]
162. Eto Y, Gao JQ, Sekiguchi F, et al. Neutralizing antibody evasion ability of adenovirus vector induced by the bioconjugation of methoxypolyethylene glycol succinimidyl propionate (MPEG-SPA). Biol Pharm Bull. 2004; 27(6):936–938. [PubMed: 15187452]
163. Eto Y, Gao JQ, Sekiguchi F, et al. PEGylated adenovirus vectors containing RGD peptides on the tip of PEG show high transduction efficiency and antibody evasion ability. J Gene Med. 2005; 7(5):604–612. [PubMed: 15543536]
164. Mok H, Palmer DJ, Ng P, Barry MA. Evaluation of polyethylene glycol modification of first-generation and helper-dependent adenoviral vectors to reduce innate immune responses. Mol Ther. 2005; 11(1):66–79. [PubMed: 15585407]
165. Croyle MA, Le HT, Linse KD, et al. PEGylated helper-dependent adenoviral vectors: highly efficient vectors with an enhanced safety profile. Gene Ther. 2005; 12(7):579–587. [PubMed: 15647765]
166. De Geest B, Snoeys J, Van Linthout S, Lievens J, Collen D. Elimination of innate immune responses and liver inflammation by PEGylation of adenoviral vectors and methylprednisolone. Hum Gene Ther. 2005; 16(12):1439–1451. [PubMed: 16390275]
167. Alemany R, Suzuki K, Curiel DT. Blood clearance rates of adenovirus type 5 in mice. J Gen Virol. 2000; 81(Pt 11):2605–2609. [PubMed: 11038370]
168. Ogawara K, Rots MG, Kok RJ, et al. A novel strategy to modify adenovirus tropism and enhance transgene delivery to activated vascular endothelial cells *in vitro* and *in vivo*. Hum Gene Ther. 2004; 15(5):433–443. [PubMed: 15144574]
169. Lanciotti J, Song A, Doukas J, et al. Targeting adenoviral vectors using heterofunctional polyethylene glycol FGF2 conjugates. Mol Ther. 2003; 8(1):99–107. [PubMed: 12842433]
170. Ogawara K, Kuldo JM, Oosterhuis K, et al. Functional inhibition of NF-κB signal transduction in αvβ3 integrin expressing endothelial cells by using RGD-PEG-modified adenovirus with a mutant *IκB* gene. Arthritis Res Ther. 2006; 8(1):R32. [PubMed: 16803639]

171. Bonsted A, Engesaeter BO, Hogset A, et al. Photochemically enhanced transduction of polymer-complexed adenovirus targeted to the epidermal growth factor receptor. J Gene Med. 2006; 8(3): 286–297. [PubMed: 16342300]

172. Croyle MA, Chirmule N, Zhang Y, Wilson JM. PEGylation of E1-deleted adenovirus vectors allows significant gene expression on readministration to liver. Hum Gene Ther. 2002; 13(15): 1887–1900. [PubMed: 12396620]

173. Wortmann A, Vohringer S, Engler T, et al. Fully detargeted polyethylene glycol-coated adenovirus vectors are potent genetic vaccines and escape from pre-existing anti-adenovirus antibodies. Mol Ther. 2008; 16(1):154–162. [PubMed: 17848961]

174. O'Riordan CR, Lachapelle A, Delgado C, et al. PEGylation of adenovirus with retention of infectivity and protection from neutralizing antibody *in vitro* and *in vivo*. Hum Gene Ther. 1999; 10(8):1349–1358. [PubMed: 10365665]

175. Hofherr SE, Shashkova EV, Weaver EA, Khare R, Barry MA. Modification of adenoviral vectors with polyethylene glycol modulates *in vivo* tissue tropism and gene expression. Mol Ther. 2008; 16(7):1276–1282. [PubMed: 18461056]

176••. Weaver EA, Barry MA. Effects of shielding adenoviral vectors with polyethylene glycol (PEG) on vector-specific and vaccine-mediated immune responses. Hum Gene Ther. 2008 (Epub ahead of print). Systematic investigation of Ad-specific and transgene product-specific immune responses following vaccination with PEGylated Ad vectors in mice. This report evaluates the impact that PEG modifications of various sizes, route of vector administration and identity of the transgene product have on the generation of Ad-neutralizing antibodies and/or T-cell response levels to transgene products.

177. Fisher KD, Stallwood Y, Green NK, et al. Polymer-coated adenovirus permits efficient retargeting and evades neutralising antibodies. Gene Ther. 2001; 8(5):341–348. [PubMed: 11313809]

178. Rihova B, Srogl J, Jelinkova M, et al. HPMA-based biodegradable hydrogels containing different forms of doxorubicin. Antitumor effects and biocompatibility. Ann NY Acad Sci. 1997; 831:57–71. [PubMed: 9616702]

179. Carlisle RC, Di Y, Cerny AM, et al. Human erythrocytes bind and inactivate type 5 adenovirus by presenting Coxsackie virus-adenovirus receptor and complement receptor 1. Blood. 2009; 113(9):1909–1918. [PubMed: 19131551]

180. Zhou QH, Miller DL, Carlisle RC, Seymour LW, Oupicky D. Ultrasound-enhanced transfection activity of HPMA-stabilized DNA polyplexes with prolonged plasma circulation. J Control Release. 2005; 106(3):416–427. [PubMed: 15967534]

181. Green NK, Morrison J, Hale S, et al. Retargeting polymer-coated adenovirus to the FGF receptor allows productive infection and mediates efficacy in a peritoneal model of human ovarian cancer. J Gene Med. 2008; 10(3):280–289. [PubMed: 18214996]

182. Green NK, Herbert CW, Hale SJ, et al. Extended plasma circulation time and decreased toxicity of polymer-coated adenovirus. Gene Ther. 2004; 11(16):1256–1263. [PubMed: 15215884]

183. Goldman CK, Rogers BE, Douglas JT, et al. Targeted gene delivery to Kaposi's sarcoma cells via the fibroblast growth factor receptor. Cancer Res. 1997; 57(8):1447–1451. [PubMed: 9108444]

184. Douglas JT, Rogers BE, Rosenfeld ME, et al. Targeted gene delivery by tropism-modified adenoviral vectors. Nat Biotechnol. 1996; 14(11):1574–1578. [PubMed: 9634824]

185. Wickham TJ, Segal DM, Roelvink PW, et al. Targeted adenovirus gene transfer to endothelial and smooth muscle cells by using bispecific antibodies. J Virol. 1996; 70(10):6831–6838. [PubMed: 8794324]

186. Tillman BW, de Gruijl TD, Luykx-de Bakker SA, et al. Maturation of dendritic cells accompanies high-efficiency gene transfer by a CD40-targeted adenoviral vector. J Immunol. 1999; 162(11): 6378–6383. [PubMed: 10352250]

187. Pereboev AV, Asiedu CK, Kawakami Y, et al. Coxsackievirus-adenovirus receptor genetically fused to anti-human CD40 scFv enhances adenoviral transduction of dendritic cells. Gene Ther. 2002; 9(17):1189–1193. [PubMed: 12170383]

188. Pereboev AV, Nagle JM, Shakhmatov MA, et al. Enhanced gene transfer to mouse dendritic cells using adenoviral vectors coated with a novel adapter molecule. Mol Ther. 2004; 9(5):712–720. [PubMed: 15120332]

189•. Huang D, Pereboev AV, Korokhov N, et al. Significant alterations of biodistribution and immune responses in Balb/c mice administered with adenovirus targeted to $CD40^+$ cells. Gene Ther. 2008; 15(4):298–308. Expansive investigation comparing the effects of administration route on relative levels of organ-specific transgene expression in mice injected with untargeted or CD40-targeted Ad5 vectors. [PubMed: 18046426]

190. Santosuosso M, Zhang X, McCormick S, et al. Mechanisms of mucosal and parenteral tuberculosis vaccinations: adenoviral-based mucosal immunization preferentially elicits sustained accumulation of immune protective CD4 and CD8 T cells within the airway lumen. J Immunol. 2005; 174(12):7986–7994. [PubMed: 15944305]

191••. Croyle MA, Patel A, Tran KN, et al. Nasal delivery of an adenovirus-based vaccine bypasses pre-existing immunity to the vaccine carrier and improves the immune response in mice. PLoS ONE. 2008; 3(10):e3548. Demonstrates that intranasal, but not intramuscular or oral, administration of an Ad5 vector encoding an Ebola antigen provides complete protection against lethal Ebola challenge in mice with Ad5 PEI. [PubMed: 18958172]

192. Patel A, Zhang Y, Croyle M, et al. Mucosal delivery of adenovirus-based vaccine protects against Ebola virus infection in mice. J Infect Dis. 2007; 196(Suppl. 2):S413–S420. [PubMed: 17940978]

193. Kiyono H, Fukuyama S. NALT- versus Peyer's-patch-mediated mucosal immunity. Nat Rev Immunol. 2004; 4(9):699–710. [PubMed: 15343369]

194. Neutra MR, Kozlowski PA. Mucosal vaccines: the promise and the challenge. Nat Rev Immunol. 2006; 6(2):148–158. [PubMed: 16491139]

195. Xiang ZQ, Gao GP, Reyes-Sandoval A, et al. Oral vaccination of mice with adenoviral vectors is not impaired by preexisting immunity to the vaccine carrier. J Virol. 2003; 77(20):10780–10789. [PubMed: 14512528]

196. Hashimoto M, Boyer JL, Hackett NR, Wilson JM, Crystal RG. Induction of protective immunity to anthrax lethal toxin with a nonhuman primate adenovirus-based vaccine in the presence of preexisting anti-human adenovirus immunity. Infect Immun. 2005; 73(10):6885–6891. [PubMed: 16177368]

197. Appaiahgari MB, Saini M, Rauthan M, Jyoti Vrati S. Immunization with recombinant adenovirus synthesizing the secretory form of Japanese encephalitis virus envelope protein protects adenovirus-exposed mice against lethal encephalitis. Microbes Infect. 2006; 8(1):92–104. [PubMed: 16126425]

198. Zhou D, Cun A, Li Y, Xiang Z, Ertl HC. A chimpanzee-origin adenovirus vector expressing the rabies virus glycoprotein as an oral vaccine against inhalation infection with rabies virus. Mol Ther. 2006; 14(5):662–672. [PubMed: 16797238]

199. Van Kampen KR, Shi Z, Gao P, et al. Safety and immunogenicity of adenovirus-vectored nasal and epicutaneous influenza vaccines in humans. Vaccine. 2005; 23(8):1029–1036. [PubMed: 15620476]

200. Lemiale F, Kong WP, Akyurek LM, et al. Enhanced mucosal immunoglobulin A response of intranasal adenoviral vector human immunodeficiency virus vaccine and localization in the central nervous system. J Virol. 2003; 77(18):10078–10087. [PubMed: 12941918]

201. Doi K, Nibu K, Ishida H, Okado H, Terashima T. Adenovirus-mediated gene transfer in olfactory epithelium and olfactory bulb: a long-term study. Ann Otol Rhinol Laryngol. 2005; 114(8):629–633. [PubMed: 16190096]

202. Damjanovic D, Zhang X, Mu J, Fe Medina M, Xing Z. Organ distribution of transgene expression following intranasal mucosal delivery of recombinant replication-defective adenovirus gene transfer vector. Genet Vaccines Ther. 2008; 6:5. [PubMed: 18261231]

203. Zabaleta A, Llopiz D, Arribillaga L, et al. Vaccination against hepatitis C virus with dendritic cells transduced with an adenovirus encoding NS3 protein. Mol Ther. 2008; 16(1):210–217. [PubMed: 17923840]

204. Proudfoot O, Apostolopoulos V, Pietersz GA. Receptor-mediated delivery of antigens to dendritic cells: anticancer applications. Mol Pharm. 2007; 4(1):58–72. [PubMed: 17228857]

205. Casimiro DR, Wang F, Schleif WA, et al. Attenuation of simian immunodeficiency virus SIVmac239 infection by prophylactic immunization with DNA and recombinant adenoviral vaccine vectors expressing Gag. J Virol. 2005; 79(24):15547–15555. [PubMed: 16306625]

206. Isman O, Roberts ML, Morgan JE, et al. Adenovirus-based targeting in myoblasts is hampered by nonhomologous vector integration. Hum Gene Ther. 2008; 19(10):1000–1008. [PubMed: 18788904]

207. Ohbayashi F, Balamotis MA, Kishimoto A, et al. Correction of chromosomal mutation and random integration in embryonic stem cells with helper-dependent adenoviral vectors. Proc Natl Acad Sci USA. 2005; 102(38):13628–13633. [PubMed: 16174752]

208. Fehse B, Roeder I. Insertional mutagenesis and clonal dominance: biological and statistical considerations. Gene Ther. 2008; 15(2):143–153. [PubMed: 17972922]

209•. Tatsis N, Fitzgerald JC, Reyes-Sandoval A, et al. Adenoviral vectors persist *in vivo* and maintain activated $CD8^+$ T cells: implications for their use as vaccines. Blood. 2007; 110(6):1916–1923. Provides evidence that low levels of persistent transgene-product expression can be detected long term in cells following vaccination with Ad vectors. In response, active effector $CD8^+$ T cells are maintained and central memory cells are created, which may offer even greater vaccine efficacy. [PubMed: 17510320]

210. Seiradake E, Henaff D, Wodrich H, et al. The cell adhesion molecule 'CAR' and sialic acid on human erythrocytes influence adenovirus *in vivo* biodistribution. PLoS Pathog. 2009; 5(1):e1000277. [PubMed: 19119424]

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301. US FDA. www.fda.gov

Key issues

- Acute toxicity and innate immune responses occurring in the presence or absence of pre-existing immunity to adenovirus (Ad) hinder the utility of Ad-based vectors for human gene therapy.
- Currently available preclinical animal models do not accurately recapitulate the human immune response to Ad vectors.
- Temporary administration of immunosuppressive drugs, as well as vector modifications, including biochemical modifications, genetic modifications, alternative and chimeric serotypes, routes of administration and cell delivery vehicles, are being investigated as methods to protect Ad vectors from pre-existing immunity and reduce vector immunogenicity.
- Unique combinations of multiple immune evasion techniques are likely to be required to develop optimized strategies for Ad vector-based approaches to be useful therapeutics that can effectively protect against or treat specific diseases, and must be carefully evaluated in stringent animal models.