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Evading the immune response upon *in vivo* gene therapy with viral vectors

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Abstract

Gene therapy has the potential to provide minimally invasive and long-term treatment for many inherited disorders that otherwise have poor prognoses and limited treatment options. The sustained therapeutic correction of genetic disease by viral gene transfer has been accomplished in patients with severe immune deficiencies, or by the transduction of an immune privileged site for the treatment of ocular disease. For other diseases and target tissues, immune responses to vectors or transgene products often present major obstacles for therapy. Innate and adaptive immunity, sometimes including preexisting or memory responses, may contribute by varying degrees to immune-mediated rejection and immunotoxicity. This review provides an overview of the immune responses to in vivo gene transfer with the most commonly used viral gene therapy vectors, and discusses strategies and protocols employed in evading the immune system in order to provide optimal gene therapy.

Keywords

Gene therapy; immune tolerance; AAV; adenovirus; lentivirus; immunity

Introduction

After the early hope and preclinical success of gene therapy were tempered by clinical setbacks, the field is beginning to witness the emergence of promising data in not only animal studies, but also in clinical trials such as those for Leber congenital amaurosis, adenosine deaminase deficiency and one type of muscular dystrophy [1–3]. Progress has been made to overcome the major technical limitations of gene therapy, such as low expression of the therapeutic gene and limited tropism, but some barriers to success still remain. One of the primary hurdles is the immune system. Mammals have evolved complex mechanisms to protect themselves against invading pathogens, including those that many viral gene-transfer vectors are derived from. Viral vectors can invoke an innate immune response via several pathways, such as the sensing of pathogen-associated molecular patterns on vector particles or in the vector genome [4]. The activation of downstream pathways can elicit antiviral and proinflammatory signals that recruit effector lymphocytes, inhibit viral transduction and stimulate the elimination of transduced cells by the priming of an adaptive immune response. Even if these initial barriers against the vector are overcome, the therapeutic transgene product may be either altered or completely absent in many of the monogenetic diseases that are typically targeted by gene therapy. For example, the *de novo*

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expression of a wild-type protein may trigger an adaptive immune response and the release of antibodies (which may be T-cell-dependent) or CTLs, or both, that mediate the destruction of transgene-expressing cells. Figure 1 outlines the sequence of interactions between the gene transfer vector, the gene products it encodes and the immune system. Although non-viral vectors, while beyond the scope of this review, may avoid some of the immune responses triggered by viral vectors, many barriers still have to be overcome with regard to efficiency of cell transduction, transgene expression and toxicity; non-viral vectors may also stimulate the innate and adaptive arm of the immune system [5]. This review highlights the immune responses to some of the most common vectors used in gene transfer protocols. In addition, concepts and strategies to circumvent or block these responses upon *in vivo* gene transfer are discussed (see Figures 2 and 3 for an overview), and specific examples are provided to illustrate their practical implementation.

Immune responses to viral vectors

Adenovirus (Ad) vectors were initially attractive for gene therapy applications because of their large packaging capacity, ability to efficiently transduce many non-dividing cell types and ease of production [6]. However, Ad vectors provoke a robust innate immune response via complement activation, and both TLR-dependent and TLR-independent mechanisms [4]. In the liver, which is the tissue most abundantly transduced following systemic administration, Ad immediately causes an increase in the expression of several proinflammatory chemokines, such as RANTES, IP-10, MIP-1β, MIP-2 and MCP-1, followed by the infiltration of neutrophils and other CD11b+ cells into the liver [7]. In addition to provoking innate immunity, pre-existing neutralizing antibodies (NABs) against the commonly used AdHu5 vector may further restrict the efficiency of gene therapy. A gutted helper-dependent Ad vector that contains no viral genes has been developed in an attempt to reduce immunogenicity; however, this vector still evokes the upregulation of many proinflammatory genes, as well as a prominent type 1 IFN response within 1 h of injection [8]. These observations from animal models were demonstrated to be accurate, if not underestimated, in 2002 in a clinical trial for the treatment of partial ornithine transcarbamylase deficiency [9]. This trial used doses of Ad deemed safe in non-human primates (NHPs) that resulted in the development of fever, myalgia, nausea, hepatotoxicity and subsequently death in 1 of the 18 patients treated [9].

Lentiviruses belong to the retrovirus family, and lentiviral-derived vectors also represent an attractive delivery platform for gene therapy because of their large packaging capacity, stable long-term transgene expression and an ability to transduce non-dividing cells. Although the low expression levels and limited tropism associated with these viruses have generally been overcome, cells transduced by lentivirus are usually eliminated within 4 to 6 weeks [10]. When injected intravenously into mice, vesicular stomatitis virus-psuedotyped lentivirus caused an increase in the expression of IFNα/β in the liver and spleen, accompanied by a rise in serum IFNα levels and followed by a decline in proviral DNA levels by 72 h post-injection [11]. In addition, IFNα/β receptor knockout mice demonstrated a 3-fold increase in transduction as well as improved persistence of lentiviral vector DNA compared with strain-matched control animals, indicating the importance of innate immunity in clearing the vector [11].

Adeno-associated virus (AAV), although limited in its packaging capacity, demonstrates a milder and more transient immune profile than other viruses used for gene therapy applications [7,8]. Immune responses to AAV vectors are known to be TLR3-independent and are at least partially dependent on Kupffer cells in the liver [12], while humoral responses against the AAV capsid are enhanced by the presence of complement [7,12,13]. In a clinical trial of an AAV-based gene therapy in patients with severe hemophilia B, two of

the seven patients treated developed what appeared to be a CTL response against the AAV capsid, as evidenced by a transient rise in the levels of liver enzymes [14]. Capsid-specific CTLs are suggested to cause the destruction of transduced hepatocytes [14,15]. This clinical observation was not predicted by any animal model and has yet to be successfully replicated in the laboratory, making the exploration of possible strategies to avoid this type of immune response challenging [16,17]. However, Li *et al* recently demonstrated the CTL-mediated killing of hepatocytes following the liver-directed gene transfer of an AAV type 2 (AAV2) vector, carrying an immunodominant epitope for chicken albumin on its surface, into mice pre-immunized with the same antigen [18]. While these data may prove useful for studying the level of CTL response elicited by different serotypes of AAV, the direct implications for the clinic may be limited as the altered transgene expression and liver toxicity fail to match that demonstrated in a hemophilia B clinical trial [14,18]. In addition, the results from two patients in this clinical trial, receiving identical doses of vector, suggested a relationship between a high titer of pre-existing NABs to the AAV2 capsid and a decrease in transduction efficiency [14]. This theory has been validated in a mouse model using pooled human serum that contained NABs to AAV2 [19]. As NABs directed against AAV2 demonstrate high levels of cross-reactivity to other AAV serotypes, and because approximately 80% of the worldwide population has circulating antibodies against AAV2 as a result of natural infection, the presence of pre-existing NABs will serve as a major hurdle in the systemic administration of AAV in the clinic [15,20].

Lowering vector doses to reduce immunogenicity

The simplest approach to restrict the interaction between a viral vector and the immune system is to increase the efficiency of the vector for gene delivery so that the amount of administered virus can be reduced. This approach can have the added benefit of producing more of the therapeutic protein, which may be beneficial for tolerance induction, particularly in the liver (see section on *Hepatic gene transfer*) [21]. One potential pitfall of this approach may be that low vector doses could be particularly susceptible to neutralization, even by low levels of NABs, an obstacle that AAV is vulnerable to given the high frequency of pre-existing immunity in the general population [20]. Improving gene therapy efficiency can be as simple as selecting an appropriate serotype for the target tissue, such as AAV8 for murine liver transduction [22]. However, interspecies differences must also be examined, as improved gene transfer with AAV8 in mice has not consistently transferred to large animal models [23].

One interesting approach has been to use balloon occlusion catheters to prevent hepatic blood outflow prior to the delivery of Ad [24]. This strategy, performed successfully in NHPs, has the benefit of localizing *in vivo* administration, while the increased hepatic pressure enhances the efficiency of vector delivery [24]. Other approaches to improving vector gene transfer have typically focused on modifying the virus capsid or genome; however, strategies to improve vector design could result in an increase in immunogenicity rather than the desired decrease.

Capsid modifications

The entry of Ad into a cell is mainly dependent on its binding to the coxsackie-adenovirus receptor (CAR), which has limited expression on the surface of many therapeutically relevant tissues. To improve the efficiency of gene transfer to tissues that lack CARs, the capsid of the Ad has been modified extensively by the genetic insertion of specific motifs, the attachment of adapter molecules and the chemical addition of polymers attached to ligands [25]. A unique combination of these methods includes polyethylene glycosylation of the Ad capsid and the attachment of an antibody that homes the vector to cells expressing E-

selectin [26]. E-selectin is specifically expressed on endothelial cells that are inflamed or undergoing rapid angiogenesis, making this approach attractive for the treatment of arthritis or cancer [26].

Maheshri *et al* have developed a directed evolution system for the AAV capsid that can potentially be used to improve vector transduction efficiency for several applications [27]. In this method, the capsid sequence is randomly mutated by PCR to produce a plasmid library of recombinant AAV capsids. These plasmids are then used to create a diverse array of AAV capsid variants that can be screened for infectivity in a desired condition, such as for the infection of resistant cell types. This method to was used to engineer an AAV capsid capable of transduction in the presence of NABs directed against AAV2 [27].

Several surface-exposed tyrosine residues on the AAV capsid were identified that were capable of being phosphorylated, leading to ubiquitination and subsequent degradation of the virus before the transgene was able to enter the cell nucleus [28]. Mutation of these tyrosine residues to phenylalanine resulted in enhanced translocation of the AAV2 vector to the nucleus, thereby avoiding proteasomal degradation in the cytoplasm. In several strains of mice, the administration of a therapeutic gene (in this case the gene for the Factor [F]IX protein), in a tyrosine-mutated vector resulted in an 8- to 17-fold increase in the expression of the FIX protein compared with a vector with a wild-type capsid [28].

Improving transgene expression

Ad vectors display robust expression, but are cleared or have reduced efficacy by pre-existing immunity, particularly when delivered systemically. An improved vector backbone (known as C4AFO) was created that contained noncoding Ad sequences, as well as human-derived ‘stuffer’ sequences [29]. Moreover, an Ad vector containing the C4AFO backbone was able to transduce muscle in mice at a increased efficiency and for prolonged periods compared with previous generation Ad vectors, even in the presence of pre-existing immunity to Ad [30].

Basic lentiviral vectors developed for gene therapy have required extensive transgene modification to achieve acceptable levels of expression; this requirement is likely to be a result of their more complex life cycle, which involves reverse transcription of the RNA genome and trafficking of the resulting DNA to the nucleus. In one study, the re-introduction of a 188bp segment of the wild-type HIV central polypurine tract, upstream of the promoter, increased transgene expression by enhancing the translocation of reverse-transcribed DNA into the nucleus [31]. Other modifications, such as the insertion of an Igκ matrix attachment region, an upstream poly-adenylation (poly-A) enhancer or post-transcriptional regulatory elements, have yielded additional improvements in transgene expression [32–34]. Lentivirus transgene expression was also improved by the insertion of an internal poly-A sequence for the transgene protein cDNA; this approach led to a 2- to 3-fold increase in transgene expression, but also reduced the viral titer [35].

AAV vectors contain a ssDNA genome that is converted to dsDNA in the nucleus, in a rate-limiting process known as second-strand synthesis. This step can be bypassed either by using a double-stranded genome or by co-expressing a phosphatase, such as PTP or PP5, that specifically inactivates FKBP52, a nuclear chaperone protein responsible for blocking second-strand synthesis [36,37].

Avoiding expression in APCs

Given the diverse tropism of most viral vectors, the imprecision of *in vivo* delivery and the wide distribution of APCs, that the transduction of APCs with gene therapy vectors is also

likely. Expression of the transgene in APCs can be detrimental to the efficacy of gene therapy, as these cells sample and present intracellular contents at the cell surface via MHC class I, and can prime an immune response against a foreign transgene product. This response has been demonstrated in murine models, where dendritic cells transduced with Ad-lacZ *ex vivo* could direct the T-cell-mediated destruction of AAV-lacZ transduced muscle cells following their adoptive transfer [38]. Furthermore, a ubiquitous or an APC-specific promoter that was used to drive the expression of a circulating protein gave rise to immune responses, whereas a liver-specific promoter demonstrated no immune response following Ad gene therapy [39].

The use of a tissue-specific promoter can avoid immunity resulting from transgene expression in APCs. Tissue-specific promoters not only reduce the immune response to the transgene product, but can also increase expression levels when compared with ubiquitous promoters. This approach has been used to modify Ad, lentivirus and AAV vectors to direct transgene expression in multiple tissues, including the liver and muscle, and for the expression of both intracellular and extracellular proteins [40–45]. However, one study demonstrated that even with the use of tissue-specific promoters, some off-target expression remained that could initiate an immune response following lentiviral gene therapy [40]. Brown *et al* employed an alternative strategy to direct expression away from APCs, by using microRNA (miRNA) to eliminate transgene expression in APCs [46]. An miRNA target sequence, complementary to an hematopoietic cell-specific miRNA target sequence, was incorporated downstream of FIX in a lentiviral vector to abolish expression in any professional, bone-marrow-derived APCs. Using this method, the stable expression of FIX in hemophilic mice during an extended period of time was demonstrated, without the development of NABs [46].

Gene therapy to immune privileged sites

Several locations within the body are known for their unique immune status and segregation from traditional immune surveillance. For example, the brain and the eye are largely isolated from the systemic circulation by tight endothelial barriers, and therefore lack the traditional lymphatics of the periphery. The liver, while not isolated from the circulation, demonstrates a relatively tolerant response to foreign antigens compared with other tissues [21,40].

(Sub) Gene therapy in the brain

The brain parenchyma is isolated from the circulation by the tight endothelial junctions of the blood-brain barrier. This barrier is advantageous for gene therapy, as it prevents antigens from escaping into the peripheral lymphatics where an adaptive immune response can be generated. Both Ad and lentivirus have been demonstrated to effectively escape an immune response in the brain below a certain dose threshold [47]. However, at higher doses, these vectors, as well as AAV, evoked an innate inflammatory response that was deleterious to gene transfer [47,48].

While pre-existing humoral immunity to the viral vector is an impediment to gene therapy in the periphery, this response should be of little consequence in the brain given its isolation from the systemic circulation. For both Ad and helper-dependent Ad, transgene expression in the brain was uninhibited in the presence of pre-existing humoral immunity to the virus [49,50]. However, following intrastriatal injection of AAV, circulating antibodies against the AAV capsid were able to completely block transduction in one study in a serotype-dependent manner [51]. Furthermore, a second injection of AAV in the opposite hemisphere of the brain increased inflammation and reduced transgene expression [47,51,52]. In addition to viral vector immunity, peripheral immunity to the transgene product can either prevent or clear transgene expression in the brain when using Ad or lentivirus vectors; this

process may involve CTLs [47,53,54]. These data have translated into clinical trials with some accuracy. For example, in a phase I clinical trial of AAV gene therapy for the treatment of Canavan disease in the brain, a dose-dependent NAB response to AAV was demonstrated [55]. However, in another phase I trial for Parkinson's disease, no correlation between transgene expression and pre-existing NABs to AAV was observed [56].

Ocular gene transfer

The eye is another immune privileged site that is characterized by a blood-tissue barrier similar to that of the brain, a lack of lymphatics, a paucity of APCs, low levels of cellular MHC class I and II expression, and an *in situ* immunosuppressive environment [57]. In addition, antigens delivered to the eye have also been demonstrated to promote peripheral tolerance to the antigen via regulatory T-cells (Tregs) [57]. Preclinical studies of gene therapy targeted to the subretinal space of the eye using AAV demonstrate minimal inflammation, with any tissue damage resulting from the physical trauma of injection [58,59]. These studies were conducted in animal models of Leber Congenital Amaurosis, a form of blindness that results from the loss of function of the *RPE65* gene. The absence of an immune response in these studies coupled with large gains in vision prompted the initiation of three clinical trials, in which AAV-RPE65 injection into the subretinal space of one eye was well tolerated with no serious adverse effects [1,60,61]. The patients in these trials also exhibited a significant improvement in visual function [1,60,61].

A recent study demonstrated that the delivery of AAV to the retina at doses that did not elicit NABs to the vector, had no effect on the subsequent transduction of the opposite eye [62]. However, an initial injection at higher doses that resulted in the production of NABs demonstrated a negative, though variable, effect on transduction of the other eye [62]. These observations, combined with the clinical safety profile of appropriately dosed subretinal AAV gene therapy, are promising for the treatment of inherited blindness disorders.

Hepatic gene transfer

The eye and the brain owe their immune privilege, at least in part, to their relative isolation from the circulation; the liver, however, may owe its immune-privileged status to a high level of circulation. The liver receives large amounts of foreign antigens and bacterial particles because this organ is directly downstream of the blood flow from the digestive tract. Most of these antigenic insults are ignored, which may explain the tolerogenic environment of the liver [63,64]. Liver-directed gene therapy has yielded long-term expression and immunological tolerance to the transgene product in animal models using Ad, lentivirus and AAV vectors, as reviewed in LoDuca *et al* [65]. The induction of transgene product-specific CD4+CD25+ Tregs, which limit antigen-specific effector T-cell functions through cell contact, cytokine-mediated and other mechanisms, is a crucial component of tolerance induction. Furthermore, Tregs induced in response to the transgene product express FoxP3 and are phenotypically comparable to naturally occurring Tregs. In addition, some transgene product-specific T-cells may be deleted or become anergic [21,66,67].

Interestingly, once tolerance had been established by the delivery of a FIX-expressing AAV (AAV-FIX) to the liver, supplementing the circulating FIX levels by vector administration to a more immunogenic site, such as skeletal muscle, was possible [68]. Similarly, tolerance to myelin basic protein, induced by gene transfer to the liver, prevented the development of experimental autoimmune encephalomyelitis in a mouse model of multiple sclerosis [69]. This concept has particular advantages in lysosomal storage diseases, where the disease manifests in both the CNS and the viscera. In a mouse model of Niemann Pick Disorder, AAV8 delivery of the therapeutic gene to the liver, followed by intracranial delivery of the

same transgene, not only avoided the humoral immune response observed with brain-only injections, but also demonstrated improved disease outcomes compared with single-site injections administered systemically or intracranially [70].

Immune suppression

Thus far, the review has focused on evading the immune system by modifying aspects of the viral vector itself or the delivery system. However, instead of hiding the vector from the immune system, another possibility is to effectively hide the immune system from the vector. This outcome can be achieved by immune suppression or by modulating the immune response away from immunity and toward tolerance.

Many of the drugs used for immunosuppression in gene therapy protocols are employed in organ transplantation. These agents function by inhibiting DNA synthesis, inhibiting cell signaling required for lymphocyte activation and proliferation, or depleting or inactivating antibodies directed against specific cell types. Typically, transient immune suppression with these drugs is preferable. For example, transient immune suppression with the glucocortocoid dexamethosone decreased the cytokine storm of the innate immune reaction, as well as the adaptive responses that generally follow the systemic administration of Ad vectors [71]. Even with the less immunogenic AAV vector, immune suppression still has benefits. For example, in canines with hemophilia, treatment with cyclophosphamide before the injection of AAV-FIX (im) yielded partial phenotypic correction and prevented an antibody response against FIX [72]. Alternatively, specific tolerance to FIX was achieved by the coadministration of the antigen, rapamycin and IL-10 prior to gene transfer [73]. A combination of the immunosuppressants mycophenolate mofetil (MMF), cyclosporin (CSA) and antithymocyte globulin in a canine model of Duchenne muscular dystrophy, demonstrated long-term expression of the therapeutic transgene, but also required immune suppression regimen of 18 weeks [74]. In this example, the aggressiveness of immune suppression may have been necessary because the muscle tissue in this disease was already inflamed, suggesting that the inflammatory state of the target tissue is important. Furthermore, care must be taken when selecting appropriate agents that do not reduce the numbers of beneficial lymphocytes, such as Tregs. For example, the use of daclizumab in an NHP study resulted in an increase in the antibody response to an AAV vector and the transgene product; this finding is likely to have been caused by daclizumab-induced depletion of tolerance-inducing CD4+CD25+ Tregs [75].

The immune response against the AAV capsid observed in the hemophilia B clinical trial prompted research into whether or not immune suppression was a viable option for human gene therapy [14]. One study using MMF and tacrolimus in NHPs [23] and a clinical trial using MMF and CSA treatment [76] suggest that the use of immune suppression has no negative impact on AAV transduction efficacy, may reduce the anti-AAV response and, at least up to a certain vector dose, may prevent CD8+ T-cell responses to the capsid [23,76]. Interestingly, the administration of rituximab plus CSA abolished antibodies against FIX resulting from AAV-FIX gene transfer in NHPs [77]. This finding could prove to be clinically relevant, as NABs to FIX may not only reduce the effectiveness of repeated administration of the gene therapy, but may also have a negative impact on the treatment of the patient.

Another potential target of immune suppression is costimulation, the interaction between an APC and a lymphocyte via a variety of cell-surface molecules that directs changes in the maturation status of one or both cells. The binding of the costimulatory molecules CD40 and CD40-ligand (CD40L) results in the maturation of the APC, allowing the APC to prime an adaptive immune response. CTLA4 (CTL antigen 4) is a costimulatory molecule expressed

on T-helper cells that sends an inhibitory signal to APCs. The administration of a soluble CTLA4-Ig fusion protein at high doses, or during an extended period, prevented immune responses following the retrovirus-mediated gene transfer of a reporter gene and a therapeutic gene in a mouse model of mucopolysaccharidosis type I (MPSI) [78,79]. Transient immune suppression with a combination of anti-CD40L and CTLA4-Ig has also been effective in generating long-term transgene expression in mouse models of MPSI and hemophilia A [79,80]. Other immune suppressive approaches include the targeting of ICOS (inducible T-cell costimulator) with mAbs and the partial blockage of signaling to T-cells with non-Fc (fragment crystallizable) receptor binding anti-CD3 molecules [81,82].

Alternative methods of immune suppression

Another interesting method of circumventing an immune response is the inclusion of certain sequences in the transgene, such as the guanine-adenine repeats of the gene encoding the EBNA-1 protein from EBV, that prevent the protein product from being displayed as an epitope [83].

The manipulation and exploitation of Tregs is also an attractive method for inducing tolerance in gene therapy. In one study, the transfer of antigen-specific Tregs mitigated the T-cell-mediated destruction of muscle cells transduced with an AAV vector expressing the highly immunogenic hemagglutinin protein [84]. The same investigators successfully grafted male mouse bone marrow cells into female recipients by the cotransfer of Tregs specific for the male antigen DBY [85]. Interestingly, the recipient mouse was not only tolerant to DBY, but also to minor antigens present on the donor bone marrow cells, including a foreign antigen expressed within the bone marrow cell, in this case EGFP (enhanced green fluorescent protein) [85].

In addition, exposure to an antigen via the mucosal route (nasal or oral) can induce tolerance to the antigen by the recruitment of Tregs, which may prove beneficial as a method to tolerize individuals prior to gene transfer [86,87]. Finally, the developing immune system may offer an environment favorable to tolerance following gene transfer either *in utero* or to the neonate [88,89].

Conclusion

Much progress remains to be made in order to be able to provide optimal gene therapy, including fine-tuning the interaction between the immune system and the viral vector used for gene transfer. Each vector, transgene product and disease present unique immunological challenges, and one approach is unlikely to be omnipotent. While animal models provide excellent preliminary data, the differences in the physiology and immunology between humans and animals necessitate that some questions must be answered in clinical trials. Which vector will prove to be the most successful in these trials remains to be determined. The immunogenicity of Ad suggests this vector may be more suitable as a vaccine or anticancer vector [90], while AAV vectors have demonstrated preclinical and some clinical success, and is considered the most viable vector for *in vivo* use in humans. However, lentivirus vectors have also demonstrated positive clinical results as an *ex vivo* gene therapy tool, and may be most useful in disease models where this approach is applicable. However, through careful study, taming or eluding the immune system sufficiently to enhance the therapeutic benefits of gene therapy seems reasonable given the vast array of emerging tools.

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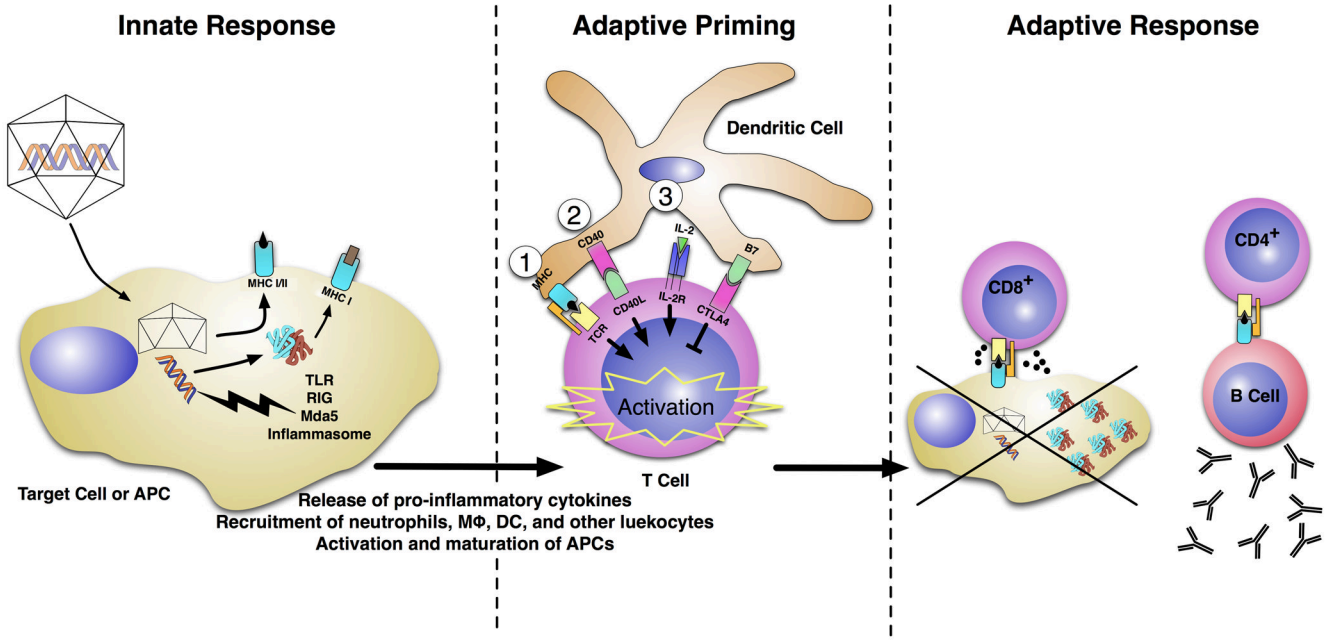

Figure 1. The immune response to viral vectors

The immune response to viral vectors occurs in three stages: innate response (A), adaptive priming (B) and adaptive response (C).

(**A**) Innate response. Upon infection of the target cell or an APC, the virus uncoats and releases its genome. At this stage, the viral genome can be recognized by TLRs, Rig-1, Mda-5 or the inflammasome. The activation of these proteins results in the release of proinflammatory cytokines, which recruit other leukocytes (including neutrophils, macrophages and dendritic cells) to the area and cause an upregulation of costimulatory molecules on APCs, leading to APC activation and maturation. Meanwhile, the capsid can be degraded and presented on MHC class I (via cross-presentation) to mark the cell for future destruction by CD8+ T-cells. Capsid epitopes can also be presented by MHC class II on infected professional APCs to initiate an antibody response that is mediated by CD4+ T-cells. The transgene protein product can also flag the cell for destruction by CD8+ T-cells via classical MHC class I presentation. APCs that have been activated by the innate response induce adaptive immunity by presenting the antigens to T-cells in the context of costimulation. (**B**) Adaptive priming. T-cells typically require three signals for activation and proliferation: (1) the antigen-specific recognition of self-MHC:peptide on the APC by the T-cell receptor (**TCR**); (2) costimulatory molecule binding at the surface of both APC and T-cells (eg, CD28:CD80/86, B7:CD28 and CD40:CD40-ligand [**CD40L**]); and (3) stimulation by growth factors, such as the cytokines IL-15, IL-12 or IL-2. Cell-surface-molecule interactions can also lead to inhibition; for example, CTL antigen 4 (**CTLA4**) binds with high affinity to B7 on the APC and sends an inhibitory signal to the T-cell. (**C**) Adaptive response. Activated effector T-cells proliferate and carry out their respective effector function. CD8+ T-cells recognize infected cells via MHC:TCR and kill them via release of cytotoxic granules containing perforin and granzymes.. CD4+ T-cells can activate plasma B-cells to produce neutralizing antibodies against the transgene product or the virus capsid.

IL-2R IL-2 receptor

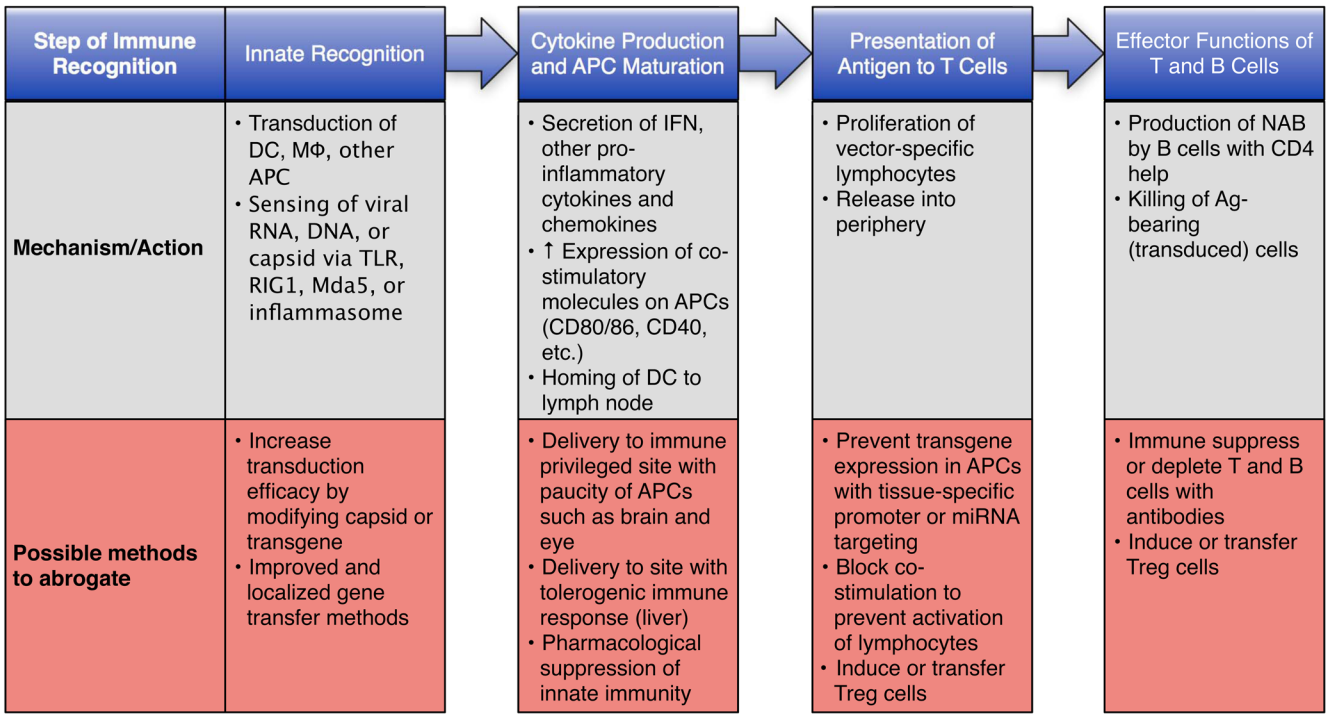

Figure 2. Strategies to avoid immune responses in gene therapy

Attempts to avoid an immune response in gene therapy can be divided into two categories: those that hide the vector and its transgene product from the immune system, and those that hide the immune system from the vector/transgene product. In either scenario, depending on the tissue and protocol, it may be possible to ultimately present such antigens to the immune system without inflammatory signals, thereby promoting immune tolerance.

miRNA microRNA, **Treg** regulatory T-cell

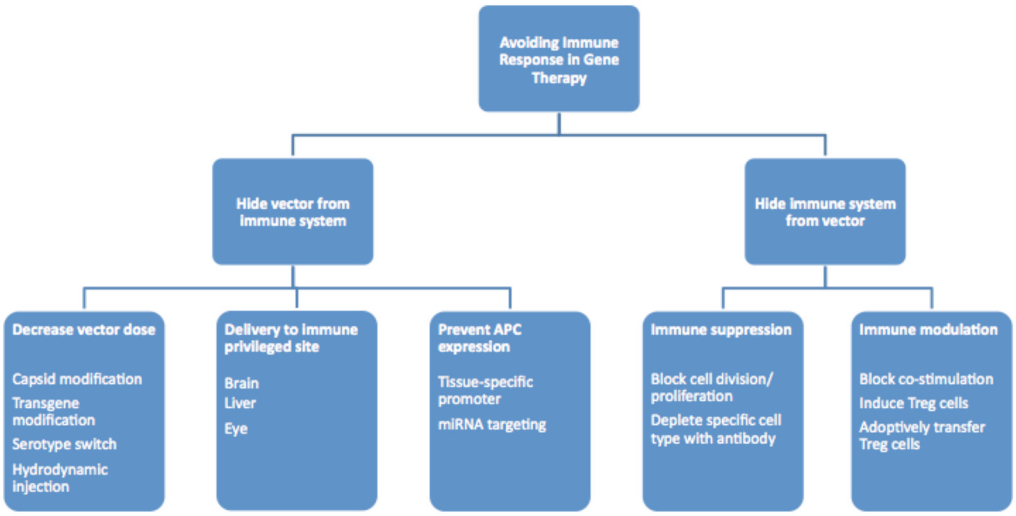

Figure 3. Steps in immune recognition and methods of intervention

The first contact the vector has with the immune system is through innate immunity, which typically results in a cytokine response within an hour. The most prolific producers of antiviral cytokines are APCs, such as plasmacytoid dendritic cells (**DC**s), conventional DCs, macrophages (**Mφ**), and B-cells. The sensing of the viral vector can occur through TLRs, Rig-1, Mda-5 or the inflammasome. These sensors, which are located in the cytoplasm, the endosome or on the cell surface, detect the vector by recognition of the viral capsid/ envelope, DNA or RNA. Cytokines and other signals that result from the ligation of these sensors induce upregulation of costimulatory molecules on the APCs, a process termed maturation. Mature DCs then home to lymphoid tissues where they present viral antigens to naïve effector T-cells (the activation requirements for APCs may be reduced in a secondary immune response as memory lymphocytes are more easily activated). T-cells that recognize a particular viral antigen become activated and proliferate, before they migrate into the periphery. These activated T-cells can then perform their respective effector function. The MHC class II presentation of peptides by B-cells to CD4+ T-cells can result in B-cell activation, which may subsequently produce antibodies against the vector or transgene product. CD8+ T-cells will specifically kill cells that present antigens via MHC class I recognition. For each step, potential countermeasures are listed.

Ag antigen, **miRNA** microRNA, **NAB** neutralizing antibody