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Review article

Liver induced transgene tolerance with AAV vectors

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ABSTRACT

Immune tolerance is a vital component of immunity, as persistent activation of immune cells causes significant tissue damage and loss of tolerance leads to autoimmunity. Likewise, unwanted immune responses can occur in inherited disorders, such as hemophilia and Pompe disease, in which patients lack any expression of protein, during treatment with enzyme replacement therapy, or gene therapy. While the liver has long been known as being tolerogenic, it was only recently appreciated in the last decade that liver directed adeno-associated virus (AAV) gene therapy can induce systemic tolerance to a transgene. In this review, we look at the mechanisms behind liver induced tolerance, discuss different factors influencing successful tolerance induction with AAV, and applications where AAV mediated tolerance may be helpful.

1. Introduction

The basis of gene therapy is the delivery of a functional copy of the disease causing gene that is missing or deficient in patients. Viruses are commonly used in the field of gene therapy for many reasons: 1) abundance, 2) easily manipulated, and 3) they have naturally evolved to deliver their genetic payload to target cells or tissues. Within the field of gene therapy, adenovirus, adeno-associated virus, retrovirus, and lentivirus have enjoyed the most success. The choice of viral vector system is based on several factors: widespread versus localized transduction, long-term vs short-term transgene expression, packaging capacity, and immunogenicity. Of all the viral based vector systems used for gene therapy, adeno-associated virus (AAV) has become one of the most commonly used today.

AAV is a parvovirus that contains a single stranded DNA genome of ~4.7 kb. AAV is unable to replicate without a helper virus and is non-pathogenic in hosts, including humans. Recombinant AAV (rAAV) retains the inverted terminal repeats (ITRs) of the wild-type genome allowing rAAV to have a packaging capacity of up to 5 kb. rAAV vectors have several important properties that make them well suited for gene therapy. They can infect non-dividing cells, vector genomes are minimally integrative and are maintained episomally, come in a wide array of serotypes with a specific tropism for a certain tissue, and importantly, have low immunogenicity [1–3]. Nevertheless, there remains a risk for immune responses. For example, when treating an inherited disorder in which there is no protein expression, the therapeutic protein can be seen as non-self and may trigger a T and B cell mediated immune response [4].

It is widely accepted that AAV liver directed gene therapy can harness the tolerogenic nature of the liver and induce systemic immunological tolerance to transgene products [5–8]. Tolerance is defined as the failure of the body to mount an immune response to an antigen whether it be to 'self' or a foreign protein. Regulatory T cells (Tregs) are known to play a crucial role in the induction and maintenance of tolerance. Tregs suppress immune responses in the periphery through a number of mechanisms including direct and indirect suppression of antigen presenting cells, B lymphocytes, and T effector cells (Fig. 1) [9–14]. By leveraging this unique ability to induce immune tolerance to transgene products, it is possible to develop lasting treatments for a multitude of diseases (for a more complete listing of diseases which have been treated using gene therapy see the article by Roncarolo et al., within this issue).

Multiple tissues have been investigated for inducing transgene tolerance including hematopoietic stem cells, thymus, muscle, and liver (for a more complete review see [15]). This review will primarily focus on AAV gene transfer to the liver, the current understanding of specific cellular interactions between resident liver and immune cells, and mechanisms of tolerance induction. We will highlight key factors to consider for successful and durable tolerance induction and provide an overview of pre-clinical data supporting AAV mediated tolerance induction in several different disease models, as well as discuss potential limitations for translation into humans. Finally, we will discuss a novel application of AAV gene therapy, using transgene tolerance induction to treat an autoimmune disease.

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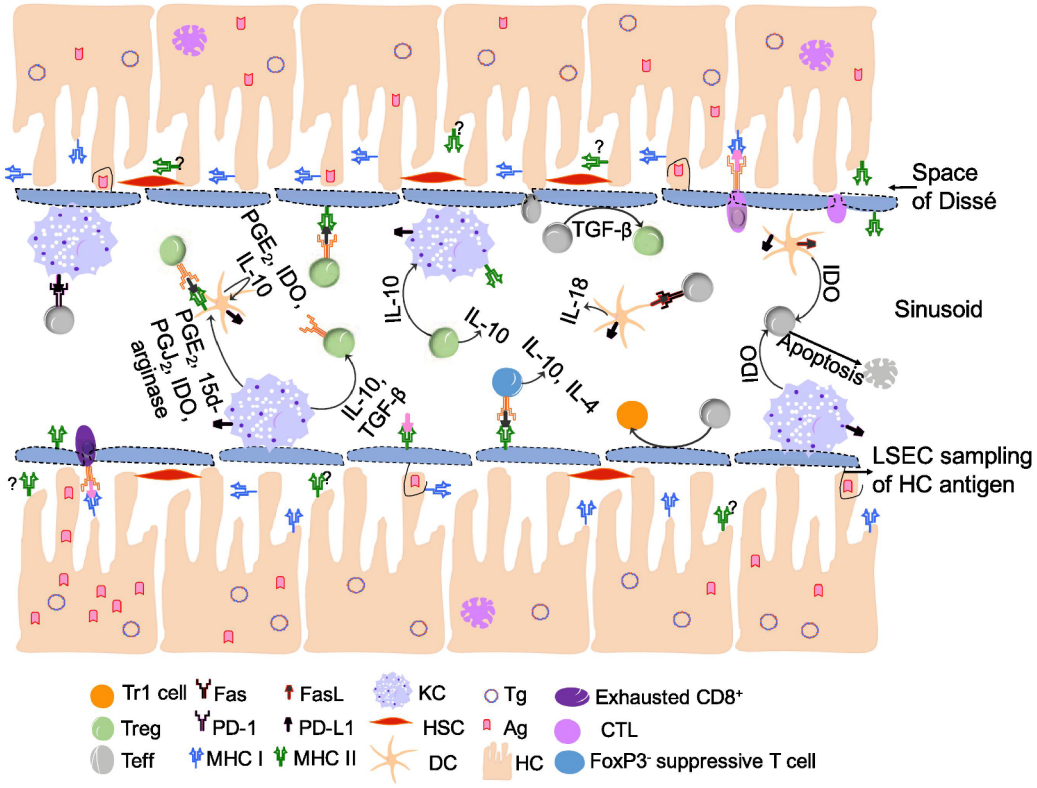

Fig. 1. Mechanisms involved in the induction of tolerance via AAV directed gene therapy. The induction of tolerance within the liver relies on the integrity of the tolerogenic environment of the liver. The maintenance of this tolerogenic environment, as well as the induction of systemic tolerance, is the result of a cellular orchestration within the liver. Tg = Transgene, Ag = Antigen, KC = Kupffer cell, HSC = Hepatic Stellate Cell, DC = Dendritic Cell, HC = Hepatocyte.

2. Immune tolerance and Tregs

A deleterious immune response to an AAV delivered transgene is a potential complication associated with long-term correction of disease. This response becomes more prominent in cases where the therapeutic gene being delivered is completely absent, not just mutated [4]. In this case, the transgene product could be recognized as non-self and trigger the activation of both humoral and cell-mediated immune responses which rely heavily on the activation of $CD4^+$ T cells for optimal effectiveness. In the case of humoral responses, $CD4^+$ T cells provide help for the maturation of B lymphocytes and production of high affinity antibodies specific for the transgene product [14]. In the case of cell-mediated responses, $CD4^+$ T cells are not directly required for the activation of $CD8^+$ responses, although, the speed and strength of the $CD8^+$ T cells activation, as well as the formation of a $CD8^+$ memory T cell response, is in fact dependent on a $CD4^+$ T cell help.

One approach for mitigating such adverse responses is through the induction of tolerance driven by transgene specific Tregs. Tregs are a unique subset of $CD4^+$ T cells expressing Forkhead box *P*3 (FoxP3) that help maintain immune homeostasis, and for this review will be defined as $CD4^+CD25^+FoxP3^+$ T cells. In addition to preventing excessive inflammatory damage to tissues, Tregs suppress self-reactive T cells that have escaped thymic selection and are considered one of the most important regulators of peripheral tolerance. Tregs are capable of suppressing other cell types in both a contact–dependent and –independent manner, through the ligation of cell surface antigens such as: cytotoxic T lymphocyte antigen 4 (CTLA-4), lymphocyte activation gene 3 (LAG-3), CD18, Neuropilin-1 (Nrp-1), lymphocyte function-associated antigen 1 (LFA-1), and CD39, or by releasing anti-inflammatory cytokines IL-10 and TGF-β, as well as perforins and granzymes [16]. As such, Tregs have the capacity to prevent immune responses in both an antigen-specific and non-specific fashion [17].

Tregs are divided into two broad categories: 1) natural Tregs (nTregs) which are developed in the thymus and 2) inducible Tregs (iTregs) which are developed from peripheral $CD4^+$ T effector cells. While functionally similar, iTregs are considered to be more plastic showing transient $FoxP3^+$ expression, while nTregs have stable $FoxP3^+$ expression [18]. Phenotypic differentiation of nTregs from iTregs can be difficult. Both Helios and Neuropilin-1 expression have been suggested as markers of nTregs [19,20], although others have argued that they are not suitable for distinguishing the two populations [21–23].

Tregs are not the only regulatory cells found within the body. Type1 regulatory (Tr1) cells are suppressive T cells that are also found within the liver. Tr1 cells are characterized as being $CD3^+CD4^+CD25^-FoxP3^-LAG3^+CD49b^+$ as well as producing IL-10 and TGF-β [24–26]. Though no conclusive link has yet been established, the fact that Tr1 cells are found within the liver and produce two cytokines common in the tolerogenic liver suggests that these cells play a role in the induction of tolerance following AAV hepatic gene transfer [27]. While there are other immune regulatory cells such as Th3 cells, $CD8^+$Tregs, and regulatory B cells, their roles in tolerance following AAV hepatic gene transfer are outside the scope of this review.

3. AAV induced liver tolerance

There is substantial evidence demonstrating that AAV hepatic gene therapy induces antigen specific Tregs that modulate immune tolerance [6,8,14,28–31]. To date, most studies supporting liver induced tolerance have utilized mouse models, especially when focusing on the mechanisms behind the induction of tolerance. Unfortunately, the genetic variances between strains of mice make understanding the process more challenging. For example, C57BL/6 mice suppress cytotoxic lymphocyte (CTL) responses to systemic AAV delivery, however the same is not true for BALB/c mice which show impaired tolerance induction and are susceptible to the clearance of transduced hepatocytes through a CTL-mediated mechanism [32]. Studies conducted in dogs

and non-human primates (NHP) show that AAV mediated liver induced tolerance is more complex and is dependent not only on the transgene, but on whether a species-specific transgene is used [33–38]. While such results are encouraging, it is currently unknown if this is true in humans.

The liver has long been known as the organ responsible for detoxifying the body of foreign antigens as well as metabolizing many drugs and nutrients. Roughly 30% of the body's total blood volume passes through the liver each minute [39]. The liver's anatomical location results in roughly 80% of the blood being delivered directly from the intestines via the portal vein and 20% coming from arteries [40]. Blood traveling from the intestines inundates the liver with nutrients, bacterial products from the gut, gram-negative bacteria (and lipopolysaccharides (LPS) found on their cell walls), and cellular debris. Yet, these potentially immunogenic antigens fail to elicit immune responses in the liver [41,42] as the liver has developed a means to locally regulate adaptive immunity.

The ability of the liver to induce tolerance was first recognized in 1969 by Calne et al. where they demonstrated that porcine liver allotransplantation was capable of preventing rejection of a secondary transplanted organ from the same donor [43]. This concept has been extended to show that reduced organ rejection is seen when cells from the organ donor are injected into the recipients liver prior to transplantation [44]. Given this unique characteristic of the liver, it is an ideal target for tolerance induction via AAV. Studies in animal models show that tolerance is dependent on classical antigen presenting cells (APCs) within the liver [45] and that restricted expression to hepatocytes enhances tolerance [5]. However, the precise mechanisms of tolerance induction via liver directed AAV gene therapy is still unclear.

The tolerogenic effects produced by the liver are controlled, in a large part, by resident APCs. Liver APCs consist of both non-conventional and conventional types. In contrast to conventional APCs, like dendritic cells (DCs), the non-conventional hepatic APCs consist of Kupffer cells (KCs), liver sinusoidal endothelial cells (LSEC), hepatocytes, and hepatic stellate cells that express low-level of major histocompatibility complex (MHC)-I/II and co-stimulatory molecules during steady state [42,46]. Although the composition of T lymphocytes found within the liver is much different compared to lymph nodes, spleen, and peripheral blood, they also play a role in the induction and maintenance of tolerance.

3.1. Kupffer cells

KCs are the largest population of resident macrophages representing between 80% and 90% of all macrophages found in the body [41,47] and ~35% of the non-parenchymal cells within the liver. KCs are responsible for the phagocytosis of particulate material, elimination of cellular debris, and clearance of pathogens from the liver. However, the life span of these cells remains unclear. Studies on this topic are conflicting with some reporting these cells are replaced as often as every 1–2 weeks [47], while others show KCs to be long-lived lasting 3 months to 1 year [48].

Unlike other macrophages in the body, KCs do not actively patrol the parenchyma scavenging for pathogens. Instead, KCs remain stationary, attached to the luminal surface of LSECs and act as an immune sentinel as they are exposed to the circulating blood. Their function is to detect, bind, and phagocytose pathogens and other foreign materials, as well as release cytokines and chemokines, thereby alerting the rest of the immune system to the presence of harmful materials [39,41,47]. At steady state, KCs are known to exist in an anti-inflammatory, M2 activation state. This may be due, in part, to the fact that under physiological conditions, KCs are exposed to a constant supply of LPS in the range of 100 pg/mL to 1 ng/mL [49]. While it may seem counter intuitive, constant LPS exposure promotes KCs to produce high amounts of IL-10, TGF-β, and arachidonic acid metabolite prostaglandin E2 (PGE_2), all of which promote an anti-inflammatory macrophage state [50–54].

In many cases, hepatic gene transfer results in the expression of a secreted transgene. KCs play an important role in the induction of tolerance to soluble antigens [55]. Recent studies have shown that KCs can drive expansion of $CD4^+CD25^+FoxP3^+$Treg cells in the liver via IL-10 [27,45,56]. They also showed that KCs were capable of further expanding Tregs by converting antigen-specific Teff into Tregs [56]. The reports went on to show that this phenomenon was only seen when KCs possessed a tolerogenic signature, which is marked by program death ligand-1 (PD-L1) expression and IL-10 production. Whereas, the loss in IL-10 resulted in a failure to convert immunogenic Teff into Tregs [56]. Others have also shown that the secretion of IL-10 is crucial for tolerance induction to hepatocyte expressed antigens [29]. It was found that the administration of AAV to the liver resulted in the production of IL-10 by KCs in response to the transgene and not the capsid. Furthermore, the authors demonstrated that IL-10 production by KCs was critical for Tregs, and the maintenance of tolerance [27,29]. These findings are paramount as most AAV tolerance induction protocols rely on hepatocytes being the cell transduced.

While KC derived IL-10 may play a major role in tolerance induction within the liver, this is not the only mechanism. At homeostasis, KCs express low amounts of both MHC I and MHC II [51]. This low MHC expression reduces the ability of the KCs to present antigen and provide signal 1 to the T cells. This incomplete activation results in either anergy or Treg induction [51]. Additionally, KCs are known to express high amounts of PGE_2, 15-deoxy$\Delta 1^{2,14}$prostaglandin J_2 (15d-PGJ_2), indoleamine 2,3-dioxygenase (IDO), and arginase [57–59]. All of which are also capable of inhibiting the induction of antigen specific T cell activation by dendritic cells [29,55,60]. Lastly, KCs have been shown to express the immune suppressive protein, PD-L1, which once bound to its receptor PD-1, induces anergy or cell death in PD-1 positive Teffs, thus promoting the expansion of Tregs [56,61].

3.2. Liver sinusoidal endothelial cells

LSECs are the most prominent non-parenchymal cells of the liver, strategically positioned in the liver, lining the sinusoid creating a physical barrier between the space of Dissè and the intraluminal space [46,62]. LSECs are the most efficient endocytic cells in the body and are responsible for the transportation of molecules from the bloodstream to hepatocyte surfaces via transcytosis [63–65]. Interestingly, LSECs have been shown to directly interact with hepatocytes, thereby acquiring hepatocyte-derived antigens and inducing Tregs [66]. This sampling of hepatocyte antigens by LSECs may prove to be an important and/or crucial step in the induction of tolerance via liver directed AAV gene therapy.

LSEC are one of the first cells within the liver to contact lymphocytes and play a role in lymphocyte recruitment. It has been shown that LSECs can present secreted antigens to $CD4^+$ T cells in such a manner that results in a tolerogenic state and not immunity [67]. Recently, Merlin et al. used a lentivirus vector to deliver clotting factor VIII (FVIII) to LSEC which resulted in the induction of tolerance in a hemophilia A mouse model [68]. The tolerogenic nature of LSECs is mediated through a verity of mechanisms. Due to high local levels of IL-10, MHC and co-stimulatory molecules CD80/CD86 expression is maintained at very low levels which is inefficient for priming of T cells [69,70]. LSECs are also capable of inducing cytokine production in T cells, but due to the lack of IL-12 priming, these $CD4^+$ T cells turn into IL-10/IL-4 producing cells [49], which were shown to have suppressive effects even though they lacked the classical Treg markers, FoxP3 and CD25 [71]. More recently, Xu et al. have shown that LSECs are responsible for inducing tolerance to auto-reactive $CD4^+$ cells by converting them into $FoxP3^-LAG^+CD49b^-$ Tr1 cells [72]. This suggests that LSEC may be responsible for the induction of Tr1 like cells as opposed to Tregs. Others have shown that LSEC are in fact capable of inducing $FoxP3^+$Tregs through the conversion of $FoxP3^-$ non-Tregs into

FoxP3$^+$Treg cells in a TGF-β dependent manner [66]. It was also found that the LSEC are in fact the most efficient cells for making this conversion when compared to DCs and KCs [66]. These findings show LSEC can induce two different regulatory cell subsets that both contribute to immunological tolerance, but further studies are needed to fully elucidate the conditions that result in each cell type, and the exact role LSECs play in the induction and maintenance of tolerance induced by liver directed AAV gene therapy.

3.3. Dendritic cells

Under steady-state conditions, there are more DCs found within the mouse liver than any other parenchymal organ [73]. DCs in the liver are found predominately in the perivascular region, portal space, beneath the Glisson's capsule, and scattered throughout the parenchyma [74]. Unlike other APCs in the liver, DCs are highly motile with migration rates from the liver being ~10^5 DCs per hour [75]. In the liver, DCs patrol the tissue and engulf antigens. However, DCs within the liver, similar to KCs, are not as efficient at presenting antigens when compared to DCs from other tissues [76–78].

In steady-state conditions, hepatic DCs have an immature (iDC) phenotype and are poor activators of Teff cells and tend to be more tolerogenic. This preference for tolerance over immunity by iDCs is due in part to their low expression of MHC II on their cell surfaces. Further, while iDCs do express the co-stimulatory molecules CD40 and CD80/86, they are at such low levels they are nearly impossible to detect. Hepatic DCs also secrete PGE_2, an ant-inflammatory prostaglandin that results in the up-regulation of IDO in DCs as well as enhances their IL-10 production, resulting in an increased capacity to induce Treg [79,80]. High levels of IL-10 and low levels of IL-12 result in an antigen-independent hypo-responsiveness that shifts Th1 cells to a Th2 phenotype, thereby reducing the likelihood of immunity and promoting development of Tregs [81,82]. IL-10 is also responsible for the expansion of Tregs as the ligation of IL-10 to its receptor on a T cell overrides co-stimulatory signals and prevents the recruitment of phosphatidylinositol 3-kinase, which has been shown to be necessary for the formation of Teff cells [83,84].

The second mechanism, through which hepatic DCs can induce or maintain tolerance, is through the direct deletion of Teff cells. In addition to a reduced expression of IL-12, tolerogenic DCs within the liver have an increased amount of IL-18 production [85]. This increased production of IL-18 results in the expression of Fas-L (CD95L, CD178) by hepatic dendritic cells. This expression of Fas-L in turn allows for the direct deletion of Teff cells through a Fas (CD95)/Fas-L manner, as Fas is constitutively expressed on Teff cells within the liver [85]. A second mechanism through which hepatic DCs induce Teff cell deletion is through a PD-L1/2/PD-1 mechanism. Within the liver, both PD-L1 and PD-L2 are expressed by DCs [86]. Simultaneously, Teff cells within the liver up-regulate PD-1 in response to constant stimulation. This allows for the deletion of PD-1 expressing Teff cells through the blockade of phosphoinositide 3-kinase (PI3K) [87]. Finally, hepatic DCs can delete Teff cells through the expression of IDO, which is secreted by predominately plasmacytoid DCs within the liver resulting in the degradation of tryptophan around Teff cells. This response is seen in conjunction with the production of toxic metabolites resulting in the apoptosis of Teff cells [88]. All of these factors show a mechanism through which DCs help promote an overall tolerogenic environment within the liver which is paramount to the success of tolerance induction via liver directed AAV gene therapy.

3.4. Hepatocytes

Hepatocytes are the most common cell type within the liver comprising ~80% of all cells. Their primary role includes metabolism, detoxification, synthesis and secretion of proteins. While it has been shown that hepatocyte restricted transgene expression enhances tolerance induction, it is unclear if hepatocytes directly or indirectly contribute to Treg induction. Hepatocytes are unique to parenchymal cells as they exhibit antigen-presenting capabilities [89,90] and circulating T cells can directly interact with hepatocytes through the fenestrations of the LSEC [91]. Under steady state conditions, hepatocytes express low amounts of MHC-I and Intercellular Adhesion Molecule 1 (ICAM-1, CD54). However, given the large size of the hepatocytes and that MHC-I and ICAM-1 are concentrated to the perisinusoidal cell membrane, it is possible for hepatocytes to prime CD8$^+$ T cells [91,92].

Under steady state conditions, hepatocytes do not have detectable surface expression of MHC-II [93,94], raising doubt in the long-held belief that the induction of tolerance following liver directed AAV gene therapy is dependent on the transduction of hepatocytes. However, MHC-II can be induced during inflammation, such as in hepatitis [89]. Even though lymphocytes can directly interact with hepatocytes through the fenestrae, it is unclear if hepatocytes directly present antigen to CD4$^+$ T cells. Burghardt et al. found that following expression of an antigen by a hepatocyte, FoxP3$^+$ Tregs were induced in a Notch-dependent pathway [95]. In work by Lüth et al., they suggest a different mechanism where induction of antigen specific Tregs was dependent on the expression of the antigen in the liver and occurred by TGF-β–dependent peripheral conversion from conventional non-Tregs [96]. While the two groups suggest different mechanisms for the induction of Tregs, there is no reason that the two are mutually exclusive. In fact, Burghardt et al. found that TGF-β significantly increased the amount of Tregs produced in their system while Lüth et al. did not look at Notch signaling. Both mechanisms may play a role in the induction of Tregs with one possibly being necessary and one being enhancing [95,96].

The induction of FoxP3$^+$ Tregs is not the only means through which hepatocytes induce tolerance. In a study using AAV hepatic gene transfer, it was found that Teff cells were directly removed in a Fas/FasL dependent manner [97]. In this work, an AAV8 vector, expressing a nuclear-targeted form of β-gal, nLacZ, was used to transduce the livers of both wild type and $Fas^{lpr/lpr}$ mice. It was found that upon AAV8 liver transduction, a significant increase of FasL was seen on hepatocytes [97]. Further, they found that in AAV8 treated mice, there was a large population of Fas expressing lymphocytes found in the liver which were missing from the naïve mice [97]. These studies suggest that AAV hepatic gene transfer can be used to induce tolerance through a multifaceted approach: the induction of antigen-specific Tregs to the transgene as well as the selective deletion of transgene specific Teff cells through a Fas/FasL dependent pathway.

4. Factors that impact the immune response to the transgene

AAV liver gene transfer results in the induction of transgene specific Tregs which can play a major role in the suppression of cellular and antibody responses and the induction of tolerance. However, there are several factors which contribute to the induction of these antigen specific Tregs, as well as the maintenance of tolerance.

4.1. A threshold level of transgene expression is required for tolerance

In the context of gene replacement therapy, the transgene needs to be expressed above a threshold level to ensure immune tolerance induction, as expression below this threshold can induce adverse immune responses [5,98]. Kumar et al., demonstrated in mice that as little as a half-log difference in vector dose resulted in the activation of a transgene-specific CD8$^+$ T cell response and clearance of transduced hepatocytes (unpublished data and [98]). Whereas, in mice receiving a sufficient tolerogenic vector dose, several overlapping pathways were found to be involved in suppressing CD8$^+$ T cell responses, including Treg, FasL, and IL-10 induction [98]. This is supported by a previous study indicating that higher transgene expression levels suppress humoral immunity and is dependent upon both Treg and Fas-FasL mediated killing of CD4$^+$ T effector cells [5].

Another factor to consider is if the transgene product is retained within the cell or secreted. Using the model antigen ovalbumin, Perrin et al. demonstrated that a log increase in vector dose was required to induce a comparable frequency of ova specific Tregs using cytoplasmic bound ova compared to secreted ova [45]. Regrettably, much of the mechanistic studies into AAV induced immune tolerance have utilized secreted transgenes. The data supports induction of both peripheral and central Tregs due, at least in part, to the fact that secreted transgenes can reach the thymus via the circulatory system [27,31,45,99]. However, non-secreted transgene products may be limited to peripheral Treg induction. More studies will be needed to fully determine if this is sufficient to regulate transgene immune responses. In support of this concept, recent work by our group demonstrated that hepatic directed AAV gene therapy can be used to induce tolerance to myelin oligodendrocyte (MOG) protein, a membrane bound protein (discussed below) suggesting that tolerance induction is not dependent on secreted transgene products [100].

4.2. AAV serotype selection

In mice, AAV8 has the highest reported natural tropism for murine hepatocytes independent of the delivery route [101,102]. Although AAV8 also has superior hepatocyte transduction in humans compared to AAV2, it may not be the best serotype for liver directed gene therapy in the clinical setting. This is supported by data from Lisowski et al. [103] and Vercauteren et al. using mice with chimeric livers comprised of human and murine hepatocytes [104]. Independently, both studies concluded that AAV3 derived from either library selection or an AAV3 variant had the highest affinity for human hepatocytes. One caveat of these studies is that chimeric mice lack other cells and tissues of human origin and may overestimate the specificity of AAV3 for human hepatocytes. Ironically, AAV3 has long been ignored as a candidate serotype due to its extremely poor transduction efficiencies in murine liver, underscoring that what works great in mouse may not always translate to other species, including humans [105].

Following AAV administration, neutralizing antibodies specific to the serotype are formed, preventing vector re-administration of the same and potentially alternative capsid serotypes [106–109]. However, a recent study demonstrated the use of a highly divergent AAV, serotype 5, is permissive to re-administration with AAV1 without the development of cross-reactive neutralizing antibodies [110]. While re-administration has been successfully performed before in non-human primates [111], this is the first study to show successful re-administration despite high titer neutralizing antibodies formed in response to the initial AAV administration. This work is promising as it demonstrates the potential for a secondary treatment is possible without the need for immune suppression. Albeit, it may preclude the use of an optimal liver targeting serotype as the primary or secondary treatment, and will likely require dose optimization for each serotype used.

4.3. Engineered and library selected capsids

Altering AAV capsids is a common technique that has been used to improve transduction efficiencies and subsequent expression levels. A seminal study by Zhong et al. found that AAV2 particles are marked for proteasomal degradation through the phosphorylation of tyrosines within the capsid [112]. A subsequent study by the same group showed that when tyrosines at positions 444, 500, and 730 were changed to phenylalanine, a 30-fold increase in transduction was seen, *in vivo* [113]. More recently, the tyrosine-phenylalanine mutated capsid was shown to result in a reduced $CD8^+$ response, a key limitation of AAV therapy [114]. Alternatively, directed evolution has also been employed to identify capsids with an increased tropism for a target tissue, or to overcome other barriers to gene therapy using capsid library screens [115–119]. The success of this technique is highlighted by the development of AAV2 variants that can withstand significantly higher neutralizing antibody concentrations [108], as well as the development of variants that have enhanced antibody evasion, requiring ~20-fold higher antibody concentrations for neutralization [120]. Such variants would result in higher transduction efficiencies in patients with pre-existing neutralizing antibodies. However, the model may bias for the selection of capsid variants that do not translate well into other species and humans [105].

Specific engineering of the AAV transgene is another technique that is used to improve expression. It has been postulated that the presence of rare codons within a transgene may result in a reduction in protein expression due to translation being slowed. Codon optimization is a technique in which codon-usage frequency in the transgene is matched to tRNA availability in the target tissue and species. This technique has proven to be beneficial in significantly increasing expression levels of FVIII, resulting in phenotypic correction of hemophilia A [121,122], without leading to toxicity or an unfolded protein response [123]. In some instances, the increased expression levels have been dramatic with a 37-fold increase seen in FVIII expression levels [124], 21-fold increase seen with hFIX [125] and a ~50-fold increase in human ornithine transcarbamylase (hOTC) expression levels in hepatocytes [126]. Recently, codon optimization has been used to correct Crigler-Najjar syndrome in Gunn rats and $UGT1A1^{-/-}$ mice at vector doses that were much lower than when non-optimized transgene was used [127–129]. However, caution must be used as current reports suggest that codon optimization may be tissue dependent [130].

4.4. Self-complementary AAV (scAAV)

Wild-type AAV and derived vectors have a single-stranded DNA (ssAAV) genome, forming DNA loops at the 5′ and 3′ ends of the inverted terminal repeats (ITR). Since the expression cassette is single-stranded DNA, synthesis of the complementary second strand is needed for mRNA transcription by DNA dependent RNA polymerase. Early studies with AAV2 vectors identified this as a rate limiting step [131]. One approach to overcome this limitation was engineered by mutation of one of the viral ITRs to direct the vector genome to package as double-stranded DNA, forming a self-complementary (scAVV) vector [132]. While the use of scAAV vectors eliminates the need for second strand synthesis and results in higher transgene levels as well as increased transgene expression levels in mice and NHP [125,133–135], the packaging capacity of scAAV is approximately half (~2.3 kb) of ssAAV which excludes many different therapeutic transgenes. However, it should be noted that increased innate immune responses, via Toll-like receptor-9, have been seen in both muscle and liver in response to the use of scAAV as compared to ssAAV, though the response seen in the liver were directed at the capsid and not the transgene [136–138]. Although there is substantial evidence showing enhanced transgene expression with scAAV2 vectors, new data suggests that the degree of enhancement may be serotype dependent. Therefore, to decrease the activation of innate immunity by TLR9 sensing of the dsDNA of scAAV, one might consider using a ssAAV vector.

5. Induction of tolerance *in utero* and in neonates

Since rAAV genomes persist episomally, most studies have focused on delivery to adult animals, to avoid dilution of vector genomes through hepatocyte expansion that occurs during development. However, certain diseases require early intervention and thus, studies have been conducted to explore the relationship between *in utero* and neonate AAV gene therapy and tolerance. One benefit to this approach is that it allows for the manipulation of the immune system at an early stage before the immune system has fully matured, thereby taking advantage of a 'window of opportunity' to induce tolerance with minimal, if any, side-effects. While this technique is less commonly utilized compared to liver directed AAV, it has enjoyed some success. In mouse and sheep pre-clinical models, it was found that *in utero* administration

of AAV was capable of inducing tolerance to the transgene [139,140]. However, there are longevity concerns associated with using AAV to induce tolerance *in utero* or in neonates. The fact that AAV genomes persist in the nucleus in concatemeric episomal forms, and does not generally integrate into the host genome [141], is compounded by AAV's inability to replicate, suggesting that as the animal or individual grows, the transgene will become increasingly more 'diluted' and ineffective over time. This concern was highlighted in a study where FIX expression in sheep was all but undetectable 6 months following *in utero* gene transfer [140]. With that said, a more recent report has shown renewed promise for AAV in this field. Hinderer et al. used a mucopolysaccharidosis type I model where AAV was administered to newborn dogs and rhesus monkeys which exhibited tolerance to the transgene without the development of neutralizing antibodies [142]. These results may provide a new avenue for *in utero* and neonatal administration of AAV for inducing tolerance.

6. Immune response to AAV transgene in other tissues

The liver is by no means the only tissue to which AAV transgenes have been delivered. Skeletal muscle has arguably been one of the most common targets of AAV gene transfer. The route of administration and the dose of the AAV can affect the immunogenicity of the vector and transgene. Intravascular and systemic routes have proved to be less immunogenic than intramuscular administration which can lead to the detection of T cell infiltrates in a dose dependent manner [143–145]. It has also been shown in a preclinical hemophilia B model that the local antigen concentration within the muscle plays a major role in the immunogenicity of the transgene [143]. In many cases, muscle gene transfer is administered in the context of neuromuscular disorders. These disorders are characterized by muscular inflammation which can affect the way the transgene is presented to the immune system thereby resulting in loss of expression instead of the sustained expression commonly seen in healthy muscle tissue [146–148]. However, there is some evidence of tolerance induction following AAV muscle gene transfer. In an rAAV1-AAT clinical trial in humans, Tregs were found in muscle biopsies up to 1 year following gene transfer and were speculated to be responsible for the control of the $CD8^+$ T cell response, as well as the continued expression of the transgene [149]. One caveat is that the AAT protein has immune modulatory properties and thus, may have contributed to local Treg infiltration in muscle. Additionally, in a clinical trial, Tregs were seen following muscle gene therapy for lipoprotein lipase deficiency, though it should be mentioned that an immunosuppressant regimen was also administered along with the AAV which may have affected the gene transfer outcome [150].

The central nervous system (CNS) has traditionally been viewed as an immune privileged organ, protected from inflammation by the blood brain barrier (BBB). However, there is evidence that the CNS is not completely protected from inflammation, as evidenced in autoimmune diseases such as multiple sclerosis [151]. It has been proven that antigen presenting cells as well as T cells are capable of crossing the BBB and infiltrating the 'protected' zone [151–154]. Several AAV serotypes have been identified or engineered to cross the BBB providing an opportunity to treat CNS diseases due to long-term expression in widespread areas of the brain, including neurons [155]. As a whole, AAV treatment within the CNS has been well tolerated as is evidenced in many clinical trials (reviewed in [156]). However, it should be noted that in large animal models, immune responses were seen in dogs and NHP when foreign or human proteins were expressed [157,158]. It is still unclear as to what is the most effective route of administration and vector dose and how these will affect immune responses to transgene following CNS targeted AAV gene therapy.

The eye is another organ that has been a popular target for AAV gene therapy with over a decade of clinical experience [159] focusing on administration to the subretinal space and the vitreous. Much like the liver and CNS, the eye is traditionally considered an immune privileged site. This 'immune privilege' is due in part to the blood-retina barrier, reduction in vascularity, and the lack of draining lymphatics. Further, antigens within the eye lead to an altered immune response, Anterior Chamber-Associated Immune Deviation. This 'less robust' immune response is characterized by TGF-β, Treg production, and skewing towards Th2 responses, leading to foreign antigen tolerance [160]. However, at least one study has seen inflammation following a dose of 1×10^{12} vg injected subretinally [161]. This finding suggests that, though the eye may be considered a tolerogenic environment, it is possible to deliver enough antigen to overcome immune deviation. The route of administration may also play a role in the immune response to AAV administered to the eye. In NHPs, it has been shown that a vector dose of 10^9 vg/eye administered intravitreal was safe with no signs of inflammation; whereas, a dose of 10^{10} vg/eye resulted in signs of inflammation in 78% of animals [162]. Similarly, a clinical study for Leber's hereditary optic neuropathy showed that inflammation was seen in response to a dose of 1.8×10^{11} vg [146,163]. These results clearly show that researchers should use caution when delivering AAV to the eye. Both, vector dose and the route of administration must be taken into consideration to avoid inflammation while also opening the possibility of reconsidering the eye as an immune privileged site.

7. Applications of AAV induced tolerance

7.1. Immunological tolerance induction to prevent or eliminate anti-drug antibodies

The need for immunological tolerance in gene therapy stems from historical data showing that patients can develop adaptive immune responses to therapeutic proteins after receiving enzyme replacement therapies. The formation of antibodies presents a challenge in enzyme replacement therapies. In the case of hemophilia, these antibodies are referred to as inhibitors and are responsible for increasing the risk of morbidity and mortality, due to break out bleeds and poor long-term control of hemostasis with bypassing agents. Hemophilia A patients, with factor VIII (FVIII) deficiency, can often successfully eliminate their inhibitors with an immune tolerance induction (ITI) protocol of high-dose and frequency administration of FVIII protein. However, hemophilia B patients with inhibitors often fail ITI due to adverse immune responses to ITI including anaphylaxis and nephrotic syndrome. Thus, the induction of tolerance via liver directed AAV gene therapy may provide an alternative approach for treating inhibitors in hemophilia B patients. To test this hypothesis, we showed that AAV8-*F9* liver gene transfer was capable of reversing pre-existing pathogenic antibodies to FIX and resulted in the long-term desensitization of anaphylaxis prone hemophilia B mice, even when protein replacement therapy was resumed [14]. In larger animal models, this finding has also been corroborated. Finn et al. showed that the administration of AAV-8 encoding FVII resulted in an initial increase in inhibitor concentrations followed by the eradication of inhibitors by day 550, in a dog model of hemophilia A [164]. In a dog model of hemophilia B, it was shown that the administration of AAV-8 FIX-Padua, a hyperfunctional form of factor 9, resulted in an initial spike in inhibitor titers at ~15 days following vector administration, followed by a decline in inhibitor titers with a complete eradication seen by day 70 [36]. In both dog studies, tolerance was confirmed by the lack of an increase in inhibitors following a re-challenge with FVII and FIX, respectively.

In a mouse model of Pompe disease, liver directed AAV was successful at preventing antibody production to the transgene, acid alpha-glucosidase (GAA) [165]. Importantly, it was also demonstrated that the antibody response remained absent following several rounds of enzyme replacement therapy [166]. However, in the case of Pompe, the liver induced tolerance is not a sufficient therapy, as protein produced in the liver is unable to adequately cross the blood brain barrier and be taken up by neuronal tissue [167]. In an attempt to circumvent this issue, Doerfler et al. have designed a co-packaged AAV9, capable of

transducing both liver and CNS, system for the simultaneous induction of tolerance and production of enzyme in neuronal tissue [168].

7.2. AAV liver gene immunotherapy for the treatment of autoimmune disorders

Another area that has garnered the attention of liver directed AAV gene therapy is in the treatment of autoimmune diseases. MS is generally considered a $CD4^+$ based autoimmune disease, caused by the breakdown of immune tolerance to specific neuroprotein antigens. Our group has shown that liver directed AAV gene therapy can restore tolerance by inducing antigen specific Tregs that target the autoimmune inflammatory response [100]. In fact, we have found that with the AAV directed hepatocyte expression of the neuroprotein myelin oligodendrocyte glycoprotein (MOG), the onset of experimental autoimmune encephalomyelitis (EAE) can be completely prevented and is robust, capable of resisting a secondary insult [100]. More significantly, we have found that when AAV is given alone, or as part of a combined immunotherapy treatment, it is capable of reversing mild to severe preexisting EAE disease [100]. This area of research is very exciting as it also opens the door for the discovery of other immune modulating therapies that may improve the efficacy of AAV at inducing tolerance.

8. Conclusion

Progress in AAV gene therapy has seen several impressive leaps in the last few decades. Preclinical studies in animal disease models have shown sustained therapeutic levels of transgene, even in disease models where the immune system normally mounts a response to the therapeutic protein. Tregs, induced by liver directed AAV gene therapy, are critical for the suppression of transgene immune responses and induction of systemic tolerance. Arguably, one of the more interesting target tissues for therapeutic transgene expression is the liver. Beyond being an ideal protein factory, the liver also modulates immune responses to dietary antigens, which is extended to gene therapy products. Although the precise mechanism is still unclear, immunological tolerance is dependent on multiple cells residing within the liver for antigen presentation and through skewing the local microenvironment towards conversion of $CD4^+$ T effector cells into Treg. A successful protocol for inducing tolerance is dependent on several critical factors. For AAV gene transfer in the liver, animal models have shown that hepatocyte restricted expression and a threshold level of transgene expression is required. In addition, other factors such as serotype selection, vector composition, dosing, and choice of immune modulation also play a role in shaping transgene immune tolerance. The field continues to move forward to develop new strategies to circumvent the anti-AAV humoral and cell mediated immunological barriers that have arisen in clinical trials. With continued safety and efficacy in treating inherited genetic disorders, it may be possible to extend AAV liver gene therapy as an immune modulatory therapy to treat autoimmune disease.

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Conflict of interest

BEH is an inventor on a pending patent related to AAV immunotherapy.

References

[1] H. Nakai, S.R. Yant, T.A. Storm, S. Fuess, L. Meuse, M.A. Kay, Extrachromosomal recombinant adeno-associated virus vector genomes are primarily responsible for stable liver transduction in vivo, J. Virol. 75 (2001) 6969–6976.
[2] A.K. Zaiss, Q. Liu, G.P. Bowen, N.C. Wong, J.S. Bartlett, D.A. Muruve, Differential activation of innate immune responses by adenovirus and adeno-associated virus vectors, J. Virol. 76 (2002) 4580–4590.
[3] A.K. Zaiss, D.A. Muruve, Immune responses to adeno-associated virus vectors, Curr. Gene Ther. 5 (2005) 323–331.
[4] O. Cao, B.E. Hoffman, B. Moghimi, S. Nayak, M. Cooper, S. Zhou, H.C. Ertl, K.A. High, R.W. Herzog, Impact of the underlying mutation and the route of vector administration on immune responses to factor IX in gene therapy for hemophilia B, Mol. Ther. 17 (2009) 1733–1742.
[5] F. Mingozzi, Y.L. Liu, E. Dobrzynski, A. Kaufhold, J.H. Liu, Y. Wang, V.R. Arruda, K.A. High, R.W. Herzog, Induction of immune tolerance to coagulation factor IX antigen by in vivo hepatic gene transfer, J. Clin. Invest. 111 (2003) 1347–1356.
[6] E. Dobrzynski, R.W. Herzog, Tolerance induction by viral in vivo gene transfer, Clin. Med. Res. 3 (2005) 234–240.
[7] E. Dobrzynski, J.C. Fitzgerald, O. Cao, F. Mingozzi, L. Wang, R.W. Herzog, Prevention of cytotoxic T lymphocyte responses to factor IX-expressing hepatocytes by gene transfer-induced regulatory T cells, Proc. Natl. Acad. Sci. U.S.A. 103 (2006) 4592–4597.
[8] O. Cao, P.A. Loduca, R.W. Herzog, Role of regulatory T cells in tolerance to coagulation factors, J. Thromb. Haemostasis 7 (Suppl. 1) (2009) 88–91.
[9] C.S. Manno, G.F. Pierce, V.R. Arruda, B. Glader, M. Ragni, J.J. Rasko, J. Rasko, M.C. Ozelo, K. Hoots, P. Blatt, B. Konkle, M. Dake, R. Kaye, M. Razavi, A. Zajko, J. Zehnder, P.K. Rustagi, H. Nakai, A. Chew, D. Leonard, J.F. Wright, R.R. Lessard, J.M. Sommer, M. Tigges, D. Sabatino, A. Luk, H. Jiang, F. Mingozzi, L. Couto, H.C. Ertl, K.A. High, M.A. Kay, Successful transduction of liver in hemophilia by AAV-Factor IX and limitations imposed by the host immune response, Nat. Med. 12 (2006) 342–347.
[10] S. Sakaguchi, T. Yamaguchi, T. Nomura, M. Ono, Regulatory T cells and immune tolerance, Cell 133 (2008) 775–787.
[11] S. Sakaguchi, K. Wing, Y. Onishi, P. Prieto-Martin, T. Yamaguchi, Regulatory T cells: how do they suppress immune responses? Int. Immunol. 21 (2009) 1105–1111.
[12] S. Sakaguchi, M. Miyara, C.M. Costantino, D.A. Hafler, FOXP3+ regulatory T cells in the human immune system, Nat. Rev. Immunol. 10 (2010) 490–500.
[13] S. Sakaguchi, Regulatory T cells: history and perspective, Methods Mol. Biol. (Clifton, N.J.) 707 (2011) 3–17.
[14] D.M. Markusic, B.E. Hoffman, G.Q. Perrin, S. Nayak, X. Wang, P.A. LoDuca, K.A. High, R.W. Herzog, Effective gene therapy for haemophilic mice with pathogenic factor IX antibodies, EMBO Mol. Med. 5 (2013) 1698–1709.
[15] B.K. Sack, R.W. Herzog, C. Terhorst, D.M. Markusic, Development of gene transfer for induction of antigen-specific tolerance, molecular therapy, Methods Clin. Development 1 (2014) 14013.
[16] S. André, D.F. Tough, S. Lacroix-Desmazes, S.V. Kaveri, J. Bayry, Surveillance of antigen-presenting cells by CD4+ CD25+ regulatory T cells in autoimmunity: immunopathogenesis and therapeutic implications, Am. J. Pathol. 174 (2009) 1575–1587.
[17] M. Caridade, L. Graca, R.M. Ribeiro, Mechanisms underlying CD4+ Treg immune regulation in the adult: from experiments to models, Front. Immunol. 4 (2013) 378.
[18] J.A. Bluestone, C.R. Mackay, J.J. O'Shea, B. Stockinger, The functional plasticity of T cell subsets, Nat. Rev. Immunol. 9 (2009) 811–816.
[19] N. Sugimoto, T. Oida, K. Hirota, K. Nakamura, T. Nomura, T. Uchiyama, S. Sakaguchi, Foxp3-dependent and -independent molecules specific for CD25+CD4+ natural regulatory T cells revealed by DNA microarray analysis, Int. Immunol. 18 (2006) 1197–1209.
[20] M. Yadav, C. Louvet, D. Davini, J.M. Gardner, M. Martinez-Llordella, S. Bailey-Bucktrout, B.A. Anthony, F.M. Sverdrup, R. Head, D.J. Kuster, P. Ruminski, D. Weiss, D. Von Schack, J.A. Bluestone, Neuropilin-1 distinguishes natural and inducible regulatory T cells among regulatory T cell subsets in vivo, J. Exp. Med. 209 (1713–1722) (2012) S1711–1719.
[21] X. Lin, M. Chen, Y. Liu, Z. Guo, X. He, D. Brand, S.G. Zheng, Advances in distinguishing natural from induced Foxp3(+) regulatory T cells, Int. J. Clin. Exp. Pathol. 6 (2013) 116–123.
[22] E. Elkord, Helios should not be cited as a marker of human thymus-derived tregs. commentary: helios(+) and helios(-) cells coexist within the natural FOXP3(+) T regulatory cell subset in humans, Front. Immunol. 7 (2016) 276.
[23] E. Szurek, A. Cebula, L. Wojciech, M. Pietrzak, G. Rempala, P. Kisielow, L. Ignatowicz, Differences in expression level of helios and neuropilin-1 do not distinguish thymus-derived from extrathymically-induced CD4+Foxp3+ regulatory T cells, PLoS One 10 (2015) e0141161.
[24] H. Zeng, R. Zhang, B. Jin, L. Chen, Type 1 regulatory T cells: a new mechanism of peripheral immune tolerance, Cell. Mol. Immunol. 12 (2015) 566–571.
[25] H. Groux, A. O'Garra, M. Bigler, M. Rouleau, S. Antonenko, J.E. de Vries, M.G. Roncarolo, A CD4+ T-cell subset inhibits antigen-specific T-cell responses and prevents colitis, Nature 389 (1997) 737–742.
[26] N. Gagliani, C.F. Magnani, S. Huber, M.E. Gianolini, M. Pala, P. Licona-Limon, B. Guo, D.R. Herbert, A. Bulfone, F. Trentini, C. Di Serio, R. Bacchetta, M. Andreani, L. Brockmann, S. Gregori, R.A. Flavell, M.G. Roncarolo, Coexpression of CD49b and LAG-3 identifies human and mouse T regulatory type 1 cells, Nat. Med. 19 (2013) 739–746.
[27] B.E. Hoffman, A.T. Martino, B.K. Sack, O. Cao, G. Liao, C. Terhorst, R.W. Herzog, Nonredundant roles of IL-10 and TGF-β in suppression of immune responses to hepatic AAV-factor IX gene transfer, Mol. Ther. 19 (2011) 1263–1272.
[28] O. Cao, E. Dobrzynski, L. Wang, S. Nayak, B. Mingle, C. Terhorst, R.W. Herzog,

Induction and role of regulatory CD4+CD25+ T cells in tolerance to the transgene product following hepatic in vivo gene transfer, Blood 110 (2007) 1132–1140.
[29] E. Breous, S. Somanathan, L.H. Vandenberghe, J.M. Wilson, Hepatic regulatory T cells and Kupffer cells are crucial mediators of systemic T cell tolerance to antigens targeting murine liver, Hepatology 50 (2009) 612–621.
[30] A.T. Martino, S. Nayak, B.E. Hoffman, M. Cooper, G. Liao, D.M. Markusic, B.J. Byrne, C. Terhorst, R.W. Herzog, Tolerance induction to cytoplasmic beta-galactosidase by hepatic AAV gene transfer: implications for antigen presentation and immunotoxicity, PLoS One 4 (2009) e6376.
[31] E. Dobrzynski, F. Mingozzi, Y.L. Liu, E. Bendo, O. Cao, L. Wang, R.W. Herzog, Induction of antigen-specific CD4+ T-cell anergy and deletion by in vivo viral gene transfer, Blood 104 (2004) 969–977.
[32] E. Breous, S. Somanathan, J.M. Wilson, BALB/c mice show impaired hepatic tolerogenic response following AAV gene transfer to the liver, Mol. Ther. 18 (2010) 766–774.
[33] J.D. Mount, R.W. Herzog, D.M. Tillson, S.A. Goodman, N. Robinson, M.L. McCleland, D. Bellinger, T.C. Nichols, V.R. Arruda, C.D. Lothrop, K.A. High, Sustained phenotypic correction of hemophilia B dogs with a factor IX null mutation by liver-directed gene therapy, Blood 99 (2002) 2670–2676.
[34] H. Jiang, D. Lillicrap, S. Patarroyo-White, T. Liu, X. Qian, C.D. Scallan, S. Powell, T. Keller, M. McMurray, A. Labelle, D. Nagy, J.A. Vargas, S. Zhou, L.B. Couto, G.F. Pierce, Multiyear therapeutic benefit of AAV serotypes 2, 6, and 8 delivering factor VIII to hemophilia A mice and dogs, Blood 108 (2006) 107–115.
[35] G.P. Niemeyer, R.W. Herzog, J. Mount, V.R. Arruda, D.M. Tillson, J. Hathcock, F.W. van Ginkel, K.A. High, C.D. Lothrop, Long-term correction of inhibitor-prone hemophilia B dogs treated with liver-directed AAV2-mediated factor IX gene therapy, Blood 113 (2009) 797–806.
[36] J.M. Crudele, J.D. Finn, J.I. Siner, N.B. Martin, G.P. Niemeyer, S. Zhou, F. Mingozzi, C.D. Lothrop, V.R. Arruda, AAV liver expression of FIX-Padua prevents and eradicates FIX inhibitor without increasing thrombogenicity in hemophilia B dogs and mice, Blood 125 (2015) 1553–1561.
[37] F. Mingozzi, N.C. Hasbrouck, E. Basner-Tschakarjan, S.A. Edmonson, D.J. Hui, D.E. Sabatino, S. Zhou, J.F. Wright, H. Jiang, G.F. Pierce, V.R. Arruda, K.A. High, Modulation of tolerance to the transgene product in a nonhuman primate model of AAV-mediated gene transfer to liver, Blood 110 (2007) 2334–2341.
[38] A.C. Nathwani, A.M. Davidoff, H. Hanawa, Y. Hu, F.A. Hoffer, A. Nikanorov, C. Slaughter, C.Y. Ng, J. Zhou, J.N. Lozier, T.D. Mandrell, E.F. Vanin, A.W. Nienhuis, Sustained high-level expression of human factor IX (hFIX) after liver-targeted delivery of recombinant adeno-associated virus encoding the hFIX gene in rhesus macaques, Blood 100 (2002) 1662–1669.
[39] K. Sheth, P. Bankey, The liver as an immune organ, Curr. Opin. Crit. Care 7 (2001).
[40] I.N. Crispe, Liver antigen-presenting cells, J. Hepatol. 54 (2011) 357–365.
[41] C.N. Jenne, P. Kubes, Immune surveillance by the liver, Nat. Immunol. 14 (2013) 996–1006.
[42] A.W. Thomson, P.A. Knolle, Antigen-presenting cell function in the tolerogenic liver environment, Nat. Rev. Immunol. 10 (2010) 753–766.
[43] R.Y. Calne, R.A. Sells, J.R. Pena, D.R. Davis, P.R. Millard, B.M. Herbertson, R.M. Binns, D.A. Davies, Induction of immunological tolerance by porcine liver allografts, Nature 223 (1969) 472–476.
[44] R.M. Gorczynski, Z. Chan, S. Chung, Z. Cohen, G. Levy, B. Sullivan, X.M. Fu, Prolongation of rat small bowel or renal allograft survival by pretransplant transfusion and/or by varying the route of allograft venous drainage, Transplantation 58 (1994) 816–820.
[45] G.Q. Perrin, I. Zolotukhin, A. Sherman, M. Biswas, Y.P. de Jong, C. Terhorst, A.M. Davidoff, R.W. Herzog, Dynamics of antigen presentation to transgene product-specific CD4(+) T cells and of Treg induction upon hepatic AAV gene transfer, Mol. Ther. Methods Clin. Development 3 (2016) 16083.
[46] A.K. Horst, K. Neumann, L. Diehl, G. Tiegs, Modulation of liver tolerance by conventional and nonconventional antigen-presenting cells and regulatory immune cells, Cell. Mol. Immunol. 13 (2016) 277–292.
[47] M. Bilzer, F. Roggel, A.L. Gerbes, Role of Kupffer cells in host defense and liver disease, Liver Int. 26 (2006) 1175–1186.
[48] L. Bouwens, M. Baekeland, R. De Zanger, E. Wisse, Quantitation, tissue distribution and proliferation kinetics of Kupffer cells in normal rat liver, Hepatology 6 (1986) 718–722.
[49] P.A. Knolle, T. Germann, U. Treichel, A. Uhrig, E. Schmitt, S. Hegenbarth, A.W. Lohse, G. Gerken, Endotoxin down-regulates T cell activation by antigen-presenting liver sinusoidal endothelial cells, J. Immunol. (Baltimore, Md.: 1950) 162 (1999) 1401–1407.
[50] P. Knolle, J. Schlaak, A. Uhrig, P. Kempf, K.H. Meyer zum Büschenfelde, G. Gerken, Human Kupffer cells secrete IL-10 in response to lipopolysaccharide (LPS) challenge, J. Hepatol. 22 (1995) 226–229.
[51] Q. You, L. Cheng, R.M. Kedl, C. Ju, Mechanism of T cell tolerance induction by murine hepatic Kupffer cells, Hepatology 48 (2008) 978–990.
[52] R.M. Bingisser, P.A. Tilbrook, P.G. Holt, U.R. Kees, Macrophage-derived nitric oxide regulates T cell activation via reversible disruption of the Jak3/STAT5 signaling pathway, J. Immunol. (Baltimore, Md.: 1950) 160 (1998) 5729–5734.
[53] P.A. Knolle, A. Uhrig, U. Protzer, M. Trippler, R. Duchmann, K.H. Meyer zum Büschenfelde, G. Gerken, Interleukin-10 expression is autoregulated at the transcriptional level in human and murine Kupffer cells, Hepatology 27 (1998) 93–99.
[54] D.M. Bissell, S.S. Wang, W.R. Jarnagin, F.J. Roll, Cell-specific expression of transforming growth factor-beta in rat liver. Evidence for autocrine regulation of hepatocyte proliferation, J. Clin. Invest. 96 (1995) 447–455.
[55] C. Ju, J.P. McCoy, C.J. Chung, M.L. Graf, L.R. Pohl, Tolerogenic role of Kupffer cells in allergic reactions, Chem. Res. Toxicol. 16 (2003) 1514–1519.
[56] F. Heymann, J. Peusquens, I. Ludwig-Portugall, M. Kohlhepp, C. Ergen, P. Niemietz, C. Martin, N. van Rooijen, J.C. Ochando, G.J. Randolph, T. Luedde, F. Ginhoux, C. Kurts, C. Trautwein, F. Tacke, Liver inflammation abrogates immunological tolerance induced by Kupffer cells, Hepatology 62 (2015) 279–291.
[57] J.S. de Bono, C.J. Logothetis, A. Molina, K. Fizazi, S. North, L. Chu, K.N. Chi, R.J. Jones, O.B. Goodman, F. Saad, J.N. Staffurth, P. Mainwaring, S. Harland, T.W. Flaig, T.E. Hutson, T. Cheng, H. Patterson, J.D. Hainsworth, C.J. Ryan, C.N. Sternberg, S.L. Ellard, A. Fléchon, M. Saleh, M. Scholz, E. Efstathiou, A. Zivi, D. Bianchini, Y. Loriot, N. Chieffo, T. Kheoh, C.M. Haqq, H.I. Scher, Abiraterone and increased survival in metastatic prostate cancer, N. Engl. J. Med. 364 (2011) 1995–2005.
[58] M.L. Yan, Y.D. Wang, Y.F. Tian, Z.D. Lai, L.N. Yan, Inhibition of allogeneic T-cell response by Kupffer cells expressing indoleamine 2,3-dioxygenase, World J. Gastroenterol. 16 (2010) 636–640.
[59] M.P. Callery, M.J. Mangino, M.W. Flye, Arginine-specific suppression of mixed lymphocyte culture reactivity by Kupffer cells–a basis of portal venous tolerance, Transplantation 51 (1991) 1076–1080.
[60] C. Wiegard, C. Frenzel, J. Herkel, K.J. Kallen, E. Schmitt, A.W. Lohse, Murine liver antigen presenting cells control suppressor activity of CD4+CD25+ regulatory T cells, Hepatology 42 (2005) 193–199.
[61] K. Wu, I. Kryczek, L. Chen, W. Zou, T.H. Welling, Kupffer cell suppression of CD8+ T cells in human hepatocellular carcinoma is mediated by B7–H1/programmed death-1 interactions, Cancer Res. 69 (2009) 8067–8075.
[62] A. Karrar, U. Broome, M. Uzunel, A.R. Qureshi, S. Sumitran-Holgersson, Human liver sinusoidal endothelial cells induce apoptosis in activated T cells: a role in tolerance induction, Gut 56 (2007) 243–252.
[63] B. Smedsrod, Clearance function of scavenger endothelial cells, Comparative Hepatol. 3 (Suppl. 1) (2004) S22.
[64] P.A. McCourt, B. Hansen, D. Svistuonov, S. Johansson, P. Longati, K. Schledzewski, J. Kzhyshkowska, S. Goerdt, B. Smedsrod, The liver sinusoidal endothelial cell hyaluronan receptor and its homolog, stabilin-1 – their roles (known and unknown) in endocytosis, Comparative Hepatol. 3 (Suppl. 1) (2004) S24.
[65] G. Kempka, V. Kolb-Bachofen, Binding, uptake, and transcytosis of ligands for mannose-specific receptors in rat liver: an electron microscopic study, Exp. Cell Res. 176 (1988) 38–48.
[66] A. Carambia, B. Freund, D. Schwinge, M. Heine, A. Laschtowitz, S. Huber, D.C. Wraith, T. Korn, C. Schramm, A.W. Lohse, J. Heeren, J. Herkel, TGF-β-dependent induction of $CD4^+CD25^+Foxp3^+$ Tregs by liver sinusoidal endothelial cells, J. Hepatol. 61 (2014) 594–599.
[67] P.A. Knolle, E. Schmitt, S. Jin, T. Germann, R. Duchmann, S. Hegenbarth, G. Gerken, A.W. Lohse, Induction of cytokine production in naive CD4(+) T cells by antigen-presenting murine liver sinusoidal endothelial cells but failure to induce differentiation toward Th1 cells, Gastroenterology 116 (1999) 1428–1440.
[68] S. Merlin, E.S. Cannizzo, E. Borroni, V. Bruscaggin, P. Schinco, W. Tulalamba, M.K. Chuah, V.R. Arruda, T. VandenDriessche, M. Prat, G. Valente, A. Follenzi, A novel platform for immune tolerance induction in hemophilia a mice, Mol. Ther. 25 (2017) 1815–1830.
[69] P.A. Knolle, A. Uhrig, S. Hegenbarth, E. Löser, E. Schmitt, G. Gerken, A.W. Lohse, IL-10 down-regulates T cell activation by antigen-presenting liver sinusoidal endothelial cells through decreased antigen uptake via the mannose receptor and lowered surface expression of accessory molecules, Clin. Exp. Immunol. 114 (1998) 427–433.
[70] A.W. Lohse, P.A. Knolle, K. Bilo, A. Uhrig, C. Waldmann, M. Ibe, E. Schmitt, G. Gerken, K.H. Meyer Zum Büschenfelde, Antigen-presenting function and B7 expression of murine sinusoidal endothelial cells and Kupffer cells, Gastroenterology 110 (1996) 1175–1181.
[71] N. Kruse, K. Neumann, A. Schrage, K. Derkow, E. Schott, U. Erben, A. Kühl, C. Loddenkemper, M. Zeitz, A. Hamann, K. Klugewitz, Priming of CD4+ T cells by liver sinusoidal endothelial cells induces CD25low forkhead box protein 3- regulatory T cells suppressing autoimmune hepatitis, Hepatology 50 (2009) 1904–1913.
[72] X. Xu, R. Jin, M. Li, K. Wang, S. Zhang, J. Hao, X. Sun, Y. Zhang, H. Wu, J. Zhang, Q. Ge, Liver sinusoidal endothelial cells induce tolerance of autoreactive CD4+ recent thymic emigrants, Sci. Rep. 6 (2016) 19861.
[73] R.J. Steptoe, R.K. Patel, V.M. Subbotin, A.W. Thomson, Comparative analysis of dendritic cell density and total number in commonly transplanted organs: morphometric estimation in normal mice, Transpl. Immunol. 8 (2000) 49–56.
[74] J. Woo, L. Lu, A.S. Rao, Y. Li, V. Subbotin, T.E. Starzl, A.W. Thomson, Isolation, phenotype, and allostimulatory activity of mouse liver dendritic cells, Transplantation 58 (1994) 484–491.
[75] K. Matsuno, T. Ezaki, S. Kudo, Y. Uehara, A life stage of particle-laden rat dendritic cells in vivo: their terminal division, active phagocytosis, and translocation from the liver to the draining lymph, J. Exp. Med. 183 (1996) 1865–1878.
[76] S. Goddard, J. Youster, E. Morgan, D.H. Adams, Interleukin-10 secretion differentiates dendritic cells from human liver and skin, Am. J. Pathol. 164 (2004) 511–519.
[77] V.G. Pillarisetty, A.B. Shah, G. Miller, J.I. Bleier, R.P. DeMatteo, Liver dendritic cells are less immunogenic than spleen dendritic cells because of differences in subtype composition, J. Immunol. (Baltimore, Md.: 1950) 172 (2004) 1009–1017.
[78] D. Tokita, T.L. Sumpter, G. Raimondi, A.F. Zahorchak, Z. Wang, A. Nakao, G.V. Mazariegos, M. Abe, A.W. Thomson, Poor allostimulatory function of liver plasmacytoid DC is associated with pro-apoptotic activity, dependent on regulatory T cells, J. Hepatol. 49 (2008) 1008–1018.
[79] D. Raïch-Regué, M. Glancy, A.W. Thomson, Regulatory dendritic cell therapy: from rodents to clinical application, Immunol. Lett. 161 (2014) 216–221.
[80] B.M. Matta, A. Castellaneta, A.W. Thomson, Tolerogenic plasmacytoid DC, Eur. J.

Immunol. 40 (2010) 2667–2676.
[81] R.A. Maldonado, U.H. von Andrian, How tolerogenic dendritic cells induce regulatory T cells, Adv. Immunol. 108 (2010) 111–165.
[82] Z.M. Bamboat, J.A. Stableford, G. Plitas, B.M. Burt, H.M. Nguyen, A.P. Welles, M. Gonen, J.W. Young, R.P. DeMatteo, Human liver dendritic cells promote T cell hyporesponsiveness, J. Immunol. (Baltimore, Md.: 1950) 182 (2009) 1901–1911.
[83] C.A. Akdis, K. Blaser, Mechanisms of interleukin-10-mediated immune suppression, Immunology 103 (2001) 131–136.
[84] R. Abu-Eid, R.N. Samara, L. Ozbun, M.Y. Abdalla, J.A. Berzofsky, K.M. Friedman, M. Mkrtichyan, S.N. Khleif, Selective inhibition of regulatory T cells by targeting the PI3K-Akt pathway, Cancer Immunol. Res. 2 (2014) 1080–1089.
[85] T. Watanabe, H. Katsukura, Y. Shirai, M. Yamori, T. Nishi, T. Chiba, T. Kita, Y. Wakatsuki, A liver tolerates a portal antigen by generating CD11c+ cells, which select Fas ligand+ Th2 cells via apoptosis, Hepatology 38 (2003) 403–412.
[86] W.W. Unger, S. Laban, F.S. Kleijwegt, A.R. van der Slik, B.O. Roep, Induction of Treg by monocyte-derived DC modulated by vitamin D3 or dexamethasone: differential role for PD-L1, Eur. J. Immunol. 39 (2009) 3147–3159.
[87] M.E. Keir, M.J. Butte, G.J. Freeman, A.H. Sharpe, PD-1 and its ligands in tolerance and immunity, Ann. Rev. Immunol. 26 (2008) 677–704.
[88] S. Schulz, A. Landi, R. Garg, J.A. Wilson, S. van Drunen, Littel-van den Hurk, Indolamine 2,3-dioxygenase expression by monocytes and dendritic cell populations in hepatitis C patients, Clin. Exp. Immunol. 180 (2015) 484–498.
[89] J. Herkel, B. Jagemann, C. Wiegard, J.F. Lazaro, S. Lueth, S. Kanzler, M. Blessing, E. Schmitt, A.W. Lohse, MHC class II-expressing hepatocytes function as antigen-presenting cells and activate specific CD4 T lymphocyutes, Hepatology 37 (2003) 1079–1085.
[90] B. Arnold, Parenchymal cells in immune and tolerance induction, Immunol. Lett. 89 (2003) 225–228.
[91] A. Warren, D.G. Le Couteur, R. Fraser, D.G. Bowen, G.W. McCaughan, P. Bertolino, T lymphocytes interact with hepatocytes through fenestrations in murine liver sinusoidal endothelial cells, Hepatology 44 (2006) 1182–1190.
[92] P. Bertolino, M.C. Trescol-Biémont, C. Rabourdin-Combe, Hepatocytes induce functional activation of naive CD8+ T lymphocytes but fail to promote survival, Eur. J. Immunol. 28 (1998) 221–236.
[93] A. Franco, V. Barnaba, P. Natali, C. Balsano, A. Musca, F. Balsano, Expression of class I and class II major histocompatibility complex antigens on human hepatocytes, Hepatology 8 (1988) 449–454.
[94] P.A. Knolle, Staying local-antigen presentation in the liver, Curr. Opin. Immunol. 40 (2016) 36–42.
[95] S. Burghardt, B. Claass, A. Erhardt, K. Karimi, G. Tiegs, Hepatocytes induce Foxp3^{+} regulatory T cells by Notch signaling, J. Leukoc Biol. 96 (2014) 571–577.
[96] S. Lüth, S. Huber, C. Schramm, T. Buch, S. Zander, C. Stadelmann, W. Brück, D.C. Wraith, J. Herkel, A.W. Lohse, Ectopic expression of neural autoantigen in mouse liver suppresses experimental autoimmune neuroinflammation by inducing antigen-specific Tregs, J. Clin. Invest. 118 (2008) 3403–3410.
[97] S.M. Faust, P. Bell, Y. Zhu, J. Sanmiguel, J.M. Wilson, The role of apoptosis in immune hyporesponsiveness following AAV8 liver gene transfer, Mol. Ther. 21 (2013) 2227–2235.
[98] S.R. Kumar, B.E. Hoffman, C. Terhorst, Y.P. de Jong, R.W. Herzog, The balance between CD8+ T cell-mediated clearance of AAV-encoded antigen in the liver and tolerance is dependent on the vector dose, Mol. Ther. 25 (2017) 880–891.
[99] R.W. Herzog, Complexity of immune responses to AAV transgene products – example of factor IX, Cell. Immunol. (2017).
[100] G.D. Keeler, S.R. Kumar, B. Palaschak, E.L. Silverberg, D.M. Markusic, N.T. Jones, B.E. Hoffman, Gene therapy induced antigen-specific Tregs inhibits neuro-inflammation and reverses disease in a mouse model of Multiple Sclerosis, Mol. Ther. (2017).
[101] M.S. Sands, AAV-mediated liver-directed gene therapy, Methods Mol. Biol. (Clifton, N.J.) 807 (2011) 141–157.
[102] M. Cooper, S. Nayak, B.E. Hoffman, C. Terhorst, O. Cao, R.W. Herzog, Improved induction of immune tolerance to factor IX by hepatic AAV-8 gene transfer, Hum. Gene Ther. 20 (2009) 767–776.
[103] L. Lisowski, A.P. Dane, K. Chu, Y. Zhang, S.C. Cunningham, E.M. Wilson, S. Nygaard, M. Grompe, I.E. Alexander, M.A. Kay, Selection and evaluation of clinically relevant AAV variants in a xenograft liver model, Nature 506 (2014) 382–386.
[104] K. Vercauteren, B.E. Hoffman, I. Zolotukhin, G.D. Keeler, J.W. Xiao, E. Basner-Tschakarjan, K.A. High, H.C. Ertl, C.M. Rice, A. Srivastava, Y.P. de Jong, R.W. Herzog, Superior in vivo transduction of human hepatocytes using engineered AAV3 capsid, Mol. Ther. 24 (2016) 1042–1049.
[105] D.M. Markusic, T.C. Nichols, E.P. Merricks, B. Palaschak, I. Zolotukhin, D. Marsic, S. Zolotukhin, A. Srivastava, R.W. Herzog, Evaluation of engineered AAV capsids for hepatic factor IX gene transfer in murine and canine models, J. Transl. Med. 15 (2017) 94.
[106] D. Grimm, J.S. Lee, L. Wang, T. Desai, B. Akache, T.A. Storm, M.A. Kay, In vitro and in vivo gene therapy vector evolution via multispecies interbreeding and retargeting of adeno-associated viruses, J. Virol. 82 (2008) 5887–5911.
[107] A. Asokan, J.C. Conway, J.L. Phillips, C. Li, J. Hegge, R. Sinnott, S. Yadav, N. DiPrimio, H.J. Nam, M. Agbandje-McKenna, S. McPhee, J. Wolff, R.J. Samulski, Reengineering a receptor footprint of adeno-associated virus enables selective and systemic gene transfer to muscle, Nat. Biotechnol. 28 (2010) 79–82.
[108] N. Maheshri, J.T. Koerber, B.K. Kaspar, D.V. Schaffer, Directed evolution of adeno-associated virus yields enhanced gene delivery vectors, Nat. Biotechnol. 24 (2006) 198–204.
[109] V.R. Arruda, W. Xiao, It's all about the clothing: capsid domination in the adeno-associated viral vector world, J. Thromb. Haemostasis 5 (2007) 12–15.
[110] A. Majowicz, D. Salas, N. Zabaleta, E. Rodriguez-Garcia, G. Gonzalez-Aseguinolaza, H. Petry, V. Ferreira, Successful repeated hepatic gene delivery in mice and non-human primates achieved by sequential administration of AAV5ch and AAV1, Mol. Ther. (2017).
[111] A.C. Nathwani, J.T. Gray, J. McIntosh, C.Y. Ng, J. Zhou, Y. Spence, M. Cochrane, E. Gray, E.G. Tuddenham, A.M. Davidoff, Safe and efficient transduction of the liver after peripheral vein infusion of self-complementary AAV vector results in stable therapeutic expression of human FIX in nonhuman primates, Blood 109 (2007) 1414–1421.
[112] L. Zhong, B. Li, G. Jayandharan, C.S. Mah, L. Govindasamy, M. Agbandje-McKenna, R.W. Herzog, K.A. Weigel-Van Aken, J.A. Hobbs, S. Zolotukhin, N. Muzyczka, A. Srivastava, Tyrosine-phosphorylation of AAV2 vectors and its consequences on viral intracellular trafficking and transgene expression, Virology 381 (2008) 194–202.
[113] L. Zhong, B. Li, C.S. Mah, L. Govindasamy, M. Agbandje-McKenna, M. Cooper, R.W. Herzog, I. Zolotukhin, K.H. Warrington, K.A. Weigel-Van Aken, J.A. Hobbs, S. Zolotukhin, N. Muzyczka, A. Srivastava, Next generation of adeno-associated virus 2 vectors: point mutations in tyrosines lead to high-efficiency transduction at lower doses, Proc. Natl. Acad. Sci. U.S.A. 105 (2008) 7827–7832.
[114] A.T. Martino, E. Basner-Tschakarjan, D.M. Markusic, J.D. Finn, C. Hinderer, S. Zhou, D.A. Ostrov, A. Srivastava, H.C. Ertl, C. Terhorst, K.A. High, F. Mingozzi, R.W. Herzog, Engineered AAV vector minimizes in vivo targeting of transduced hepatocytes by capsid-specific CD8+ T cells, Blood 121 (2013) 2224–2233.
[115] H. Büning, A. Huber, L. Zhang, N. Meumann, U. Hacker, Engineering the AAV capsid to optimize vector-host-interactions, Curr. Opin. Pharmacol. 24 (2015) 94–104.
[116] D. Marsic, L. Govindasamy, S. Currlin, D.M. Markusic, Y.S. Tseng, R.W. Herzog, M. Agbandje-McKenna, S. Zolotukhin, Vector design Tour de Force: integrating combinatorial and rational approaches to derive novel adeno-associated virus variants, Mol. Ther. 22 (2014) 1900–1909.
[117] K.J. Excoffon, J.T. Koerber, D.D. Dickey, M. Murtha, S. Keshavjee, B.K. Kaspar, J. Zabner, D.V. Schaffer, Directed evolution of adeno-associated virus to an infectious respiratory virus, Proc. Natl. Acad. Sci. U.S.A. 106 (2009) 3865–3870.
[118] J.T. Koerber, J.H. Jang, D.V. Schaffer, DNA shuffling of adeno-associated virus yields functionally diverse viral progeny, Mol. Ther. 16 (2008) 1703–1709.
[119] C. Li, S. Wu, B. Albright, M. Hirsch, W. Li, Y.S. Tseng, M. Agbandje-McKenna, S. McPhee, A. Asokan, R.J. Samulski, Development of patient-specific AAV vectors after neutralizing antibody selection for enhanced muscle gene transfer, Mol. Ther. 24 (2016) 53–65.
[120] M.A. Bartel, J.R. Weinstein, D.V. Schaffer, Directed evolution of novel adeno-associated viruses for therapeutic gene delivery, Gene Ther. 19 (2012) 694–700.
[121] B.K. Sack, S. Merchant, D.M. Markusic, A.C. Nathwani, A.M. Davidoff, B.J. Byrne, R.W. Herzog, Transient B cell depletion or improved transgene expression by codon optimization promote tolerance to factor VIII in gene therapy, PLoS One 7 (2012) e37671.
[122] J. McIntosh, P.J. Lenting, C. Rosales, D. Lee, S. Rabbanian, D. Raj, N. Patel, E.G. Tuddenham, O.D. Christophe, J.H. McVey, S. Waddington, A.W. Nienhuis, J.T. Gray, P. Fagone, F. Mingozzi, S.Z. Zhou, K.A. High, M. Cancio, C.Y. Ng, J. Zhou, C.L. Morton, A.M. Davidoff, A.C. Nathwani, Therapeutic levels of FVIII following a single peripheral vein administration of rAAV vector encoding a novel human factor VIII variant, Blood 121 (2013) 3335–3344.
[123] I. Zolotukhin, D.M. Markusic, B. Palaschak, B.E. Hoffman, M.A. Srikanthan, R.W. Herzog, Potential for cellular stress response to hepatic factor VIII expression from AAV vector, molecular therapy, Methods Clin. Develop. 3 (2016) 16063.
[124] C. Binny, J. McIntosh, M. Della Peruta, H. Kymalainen, E.G. Tuddenham, S.M. Buckley, S.N. Waddington, J.H. McVey, Y. Spence, C.L. Morton, A.J. Thrasher, J.T. Gray, F.J. Castellino, A.F. Tarantal, A.M. Davidoff, A.C. Nathwani, AAV-mediated gene transfer in the perinatal period results in expression of FVII at levels that protect against fatal spontaneous hemorrhage, Blood 119 (2012) 957–966.
[125] Z. Wu, J. Sun, T. Zhang, C. Yin, F. Yin, T. Van Dyke, R.J. Samulski, P.E. Monahan, Optimization of self-complementary AAV vectors for liver-directed expression results in sustained correction of hemophilia B at low vector dose, Mol. Ther. 16 (2008) 280–289.
[126] P. Bell, L. Wang, S.J. Chen, H. Yu, Y. Zhu, M. Nayal, Z. He, J. White, D. Lebel-Hagan, J.M. Wilson, Effects of Self-complementarity, codon optimization, transgene, and dose on liver transduction with AAV8, Hum. Gene Ther. Methods 27 (2016) 228–237.
[127] G. Ronzitti, G. Bortolussi, R. van Dijk, F. Collaud, S. Charles, C. Leborgne, P. Vidal, S. Martin, B. Gjata, M.S. Sola, L. van Wittenberghe, A. Vignaud, P. Veron, P.J. Bosma, A.F. Muro, F. Mingozzi, A translationally optimized AAV-UGT1A1 vector drives safe and long-lasting correction of Crigler-Najjar syndrome, molecular therapy, Methods Clin. Develop. 3 (2016) 16049.
[128] J. Seppen, C. Bakker, B. de Jong, C. Kunne, K. van den Oever, K. Vandenberghe, R. de Waart, J. Twisk, P. Bosma, Adeno-associated virus vector serotypes mediate sustained correction of bilirubin UDP glucuronosyltransferase deficiency in rats, Mol. Ther. 13 (2006) 1085–1092.
[129] G. Bortolussi, L. Zentillin, J. Vaníkova, L. Bockor, C. Bellarosa, A. Mancarella, E. Vianello, C. Tiribelli, M. Giacca, L. Vitek, A.F. Muro, Life-long correction of hyperbilirubinemia with a neonatal liver-specific AAV-mediated gene transfer in a lethal mouse model of Crigler-Najjar Syndrome, Hum. Gene Ther. 25 (2014) 844–855.
[130] H.C. Brown, P.M. Zakas, S.N. George, E.T. Parker, H.T. Spencer, C.B. Doering, Tissue-directed transgene engineering for AAV and lentivector gene therapy approaches, Mol. Ther. 24 (2016) S287.
[131] F.K. Ferrari, T. Samulski, T. Shenk, R.J. Samulski, Second-strand synthesis is a

rate-limiting step for efficient transduction by recombinant adeno-associated virus vectors, J. Virol. 70 (1996) 3227–3234.
[132] D.M. McCarty, P.E. Monahan, R.J. Samulski, Self-complementary recombinant adeno-associated virus (scAAV) vectors promote efficient transduction independently of DNA synthesis, Gene Ther. 8 (2001) 1248–1254.
[133] D.M. McCarty, H. Fu, P.E. Monahan, C.E. Toulson, P. Naik, R.J. Samulski, Adeno-associated virus terminal repeat (TR) mutant generates self-complementary vectors to overcome the rate-limiting step to transduction in vivo, Gene Ther. 10 (2003) 2112–2118.
[134] A.C. Nathwani, C. Rosales, J. McIntosh, G. Rastegarlari, D. Nathwani, D. Raj, S. Nawathe, S.N. Waddington, R. Bronson, S. Jackson, R.E. Donahue, K.A. High, F. Mingozzi, C.Y. Ng, J. Zhou, Y. Spence, M.B. McCarville, M. Valentine, J. Allay, J. Coleman, S. Sleep, J.T. Gray, A.W. Nienhuis, A.M. Davidoff, Long-term safety and efficacy following systemic administration of a self-complementary AAV vector encoding human FIX pseudotyped with serotype 5 and 8 capsid proteins, Mol. Ther. 19 (2011) 876–885.
[135] D. Raj, A.M. Davidoff, A.C. Nathwani, Self-complementary adeno-associated viral vectors for gene therapy of hemophilia B: progress and challenges, Expert Rev. Hematol. 4 (2011) 539–549.
[136] J. Zhu, X. Huang, Y. Yang, The TLR9-MyD88 pathway is critical for adaptive immune responses to adeno-associated virus gene therapy vectors in mice, J. Clin. Invest. 119 (2009) 2388–2398.
[137] A.T. Martino, M. Suzuki, D.M. Markusic, I. Zolotukhin, R.C. Ryals, B. Moghimi, H.C. Ertl, D.A. Muruve, B. Lee, R.W. Herzog, The genome of self-complementary adeno-associated viral vectors increases Toll-like receptor 9-dependent innate immune responses in the liver, Blood 117 (2011) 6459–6468.
[138] T. Wu, K. Töpfer, S.W. Lin, H. Li, A. Bian, X.Y. Zhou, K.A. High, H.C. Ertl, Self-complementary AAVs induce more potent transgene product-specific immune responses compared to a single-stranded genome, Mol. Ther. 20 (2012) 572–579.
[139] D.E. Sabatino, T.C. MacKenzie, W. Peranteau, S. Edmonson, C. Campagnoli, Y.-L. Liu, A.W. Flake, K.A. High, Persistent expression of hF.IX after tolerance induction by in utero or neonatal administration of AAV-1-F.IX in hemophilia B mice, Mol. Ther. 15 (2007) 1677–1685.
[140] A.L. David, J. McIntosh, D.M. Peebles, T. Cook, S. Waddington, B. Weisz, V. Wigley, K. Abi-Nader, M. Boyd, A.M. Davidoff, A.C. Nathwani, Recombinant adeno-associated virus-mediated in utero gene transfer gives therapeutic transgene expression in the sheep, Hum. Gene Ther. 22 (2011) 419–426.
[141] M. Penaud-Budloo, C. Le Guiner, A. Nowrouzi, A. Toromanoff, Y. Cherel, P. Chenuaud, M. Schmidt, C. von Kalle, F. Rolling, P. Moullier, R.O. Snyder, Adeno-associated virus vector genomes persist as episomal chromatin in primate muscle, J. Virol. 82 (2008) 7875–7885.
[142] C. Hinderer, P. Bell, J.P. Louboutin, Y. Zhu, H. Yu, G. Lin, R. Choa, B.L. Gurda, J. Bagel, P. O'Donnell, T. Sikora, T. Ruane, P. Wang, A.F. Tarantal, M.L. Casal, M.E. Haskins, J.M. Wilson, Neonatal systemic AAV induces tolerance to CNS gene therapy in MPS I dogs and nonhuman primates, Mol. Ther. 23 (2015) 1298–1307.
[143] R.W. Herzog, P.A. Fields, V.R. Arruda, J.O. Brubaker, E. Armstrong, D. McClintock, D.A. Bellinger, L.B. Couto, T.C. Nichols, K.A. High, Influence of vector dose on factor IX-specific T and B cell responses in muscle-directed gene therapy, Hum. Gene Ther. 13 (2002) 1281–1291.
[144] A. Toromanoff, O. Adjali, T. Larcher, M. Hill, L. Guigand, P. Chenuaud, J.Y. Deschamps, O. Gauthier, G. Blancho, B. Vanhove, F. Rolling, Y. Chérel, P. Moullier, I. Anegon, C. Le Guiner, Lack of immunotoxicity after regional intravenous (RI) delivery of rAAV to nonhuman primate skeletal muscle, Mol. Ther. 18 (2010) 151–160.
[145] P. Matzinger, The danger model: a renewed sense of self, Science 296 (2002) 301–305.
[146] F. Mingozzi, K.A. High, Overcoming the host immune response to adeno-associated virus gene delivery vectors: the race between clearance, tolerance, neutralization, and escape, Annu. Rev. Virol. 4 (2017) 511–534.
[147] K.M. Flanigan, K. Campbell, L. Viollet, W. Wang, A.M. Gomez, C.M. Walker, J.R. Mendell, Anti-dystrophin T cell responses in Duchenne muscular dystrophy: prevalence and a glucocorticoid treatment effect, Hum. Gene Ther. 24 (2013) 797–806.
[148] J.R. Mendell, K. Campbell, L. Rodino-Klapac, Z. Sahenk, C. Shilling, S. Lewis, D. Bowles, S. Gray, C. Li, G. Galloway, V. Malik, B. Coley, K.R. Clark, J. Li, X. Xiao, J. Samulski, S.W. McPhee, R.J. Samulski, C.M. Walker, Dystrophin immunity in Duchenne's muscular dystrophy, N. Engl. J. Med. 363 (2010) 1429–1437.
[149] C. Mueller, J.D. Chulay, B.C. Trapnell, M. Humphries, B. Carey, R.A. Sandhaus, N. G. McElvaney, L. Messina, Q. Tang, F.N. Rouhani, M. Campbell-Thompson, A.D. Fu, A. Yachnis, D.R. Knop, G.-j. Ye, M. Brantly, R. Calcedo, S. Somanathan, L.P. Richman, R.H. Vonderheide, M.A. Hulme, T.M. Brusko, J.M. Wilson, T.R. Flotte, Human Treg responses allow sustained recombinant adeno-associated virus–mediated transgene expression, 2013.
[150] V. Ferreira, J. Twisk, K. Kwikkers, E. Aronica, D. Brisson, J. Methot, H. Petry, D. Gaudet, Immune responses to intramuscular administration of alipogene tiparvovec (AAV1-LPL(S447X)) in a phase II clinical trial of lipoprotein lipase deficiency gene therapy, Hum. Gene Ther. 25 (2014) 180–188.
[151] M. Schwartz, A. Deczkowska, Neurological disease as a failure of brain-immune crosstalk: the multiple faces of neuroinflammation, Trends Immunol. 37 (2016) 668–679.
[152] M.A. Lopes Pinheiro, G. Kooij, M.R. Mizee, A. Kamermans, G. Enzmann, R. Lyck, M. Schwaninger, B. Engelhardt, H.E. de Vries, Immune cell trafficking across the barriers of the central nervous system in multiple sclerosis and stroke, Biochim. Biophys. Acta 2016 (1862) 461–471.
[153] C. Coisne, R. Lyck, B. Engelhardt, Live cell imaging techniques to study T cell trafficking across the blood-brain barrier in vitro and in vivo, Fluids Barriers CNS 10 (2013) 7.
[154] D. Sagar, C. Foss, R. El Baz, M.G. Pomper, Z.K. Khan, P. Jain, Mechanisms of dendritic cell trafficking across the blood-brain barrier, J. Neuroimmune Pharmacol. 7 (2012) 74–94.
[155] S. Duque, B. Joussemet, C. Riviere, T. Marais, L. Dubreil, A.M. Douar, J. Fyfe, P. Moullier, M.A. Colle, M. Barkats, Intravenous administration of self-complementary AAV9 enables transgene delivery to adult motor neurons, Mol. Ther. 17 (2009) 1187–1196.
[156] M. Hocquemiller, L. Giersch, M. Audrain, S. Parker, N. Cartier, Adeno-associated virus-based gene therapy for CNS diseases, Hum. Gene Ther. 27 (2016) 478–496.
[157] V. Haurigot, S. Marco, A. Ribera, M. Garcia, A. Ruzo, P. Villacampa, E. Ayuso, S. Anor, A. Andaluz, M. Pineda, G. Garcia-Fructuoso, M. Molas, L. Maggioni, S. Munoz, S. Motas, J. Ruberte, F. Mingozzi, M. Pumarola, F. Bosch, Whole body correction of mucopolysaccharidosis IIIA by intracerebrospinal fluid gene therapy, J. Clin. Invest. (2013).
[158] L. Samaranch, W.S. Sebastian, A.P. Kells, E.A. Salegio, G. Heller, J.R. Bringas, P. Pivirotto, S. DeArmond, J. Forsayeth, K.S. Bankiewicz, AAV9-mediated expression of a non-self protein in nonhuman primate central nervous system triggers widespread neuroinflammation driven by antigen-presenting cell transduction, Mol. Ther. 22 (2014) 329–337.
[159] N.A. Moore, N. Morral, T.A. Ciulla, P. Bracha, Gene therapy for inherited retinal and optic nerve degenerations, Expert Opin. Biol. Ther. (2017) 1–13.
[160] A.W. Taylor, Ocular immune privilege, Eye 23 (2009).
[161] J.W. Bainbridge, M.S. Mehat, V. Sundaram, S.J. Robbie, S.E. Barker, C. Ripamonti, A. Georgiadis, F.M. Mowat, S.G. Beattie, P.J. Gardner, K.L. Feathers, V.A. Luong, S. Yzer, K. Balaggan, A. Viswanathan, T.J. de Ravel, I. Casteels, G.E. Holder, N. Tyler, F.W. Fitzke, R.G. Weleber, M. Nardini, A.T. Moore, D.A. Thompson, S.M. Petersen-Jones, M. Michaelides, L.I. van den Born, A. Stockman, A.J. Smith, G. Rubin, R.R. Ali, Long-term effect of gene therapy on Leber's congenital amaurosis, N. Engl. J. Med. 372 (2015) 1887–1897.
[162] T.K. Maclachlan, M. Lukason, M. Collins, R. Munger, E. Isenberger, C. Rogers, S. Malatos, E. Dufresne, J. Morris, R. Calcedo, G. Veres, A. Scaria, L. Andrews, S. Wadsworth, Preclinical safety evaluation of AAV2-sFLT01- a gene therapy for age-related macular degeneration, Mol. Ther. 19 (2011) 326–334.
[163] S. Uretsky, J. Sahel, A. Galy, N. Thomasson, G. Honnet, et al., A recombinant AAV2/2 carrying the wild-type ND4 gene for the treatment of LHON: preliminary results of a first-in-man study and upcoming pivotal efficacy trials, in: 12th International Symposium on Ocular Pharmacology and TherapeuticsBerlin, 2016.
[164] J.D. Finn, M.C. Ozelo, D.E. Sabatino, H.W. Franck, E.P. Merricks, J.M. Crudele, S. Zhou, H.H. Kazazian, D. Lillicrap, T.C. Nichols, V.R. Arruda, Eradication of neutralizing antibodies to factor VIII in canine hemophilia A after liver gene therapy, Blood 116 (2010) 5842–5848.
[165] L.M. Franco, B. Sun, X. Yang, A. Bird, H. Zhang, A. Schneider, T. Brown, S.P. Young, T.M. Clay, A. Amalfitano, Y.T. Chen, D.D. Koeberl, Evasion of immune responses to introduced human acid alpha-glucosidase by liver-restricted expression in glycogen storage disease type II, Mol. Ther. 12 (2005) 876–884.
[166] B. Sun, A. Bird, S.P. Young, P.S. Kishnani, Y.T. Chen, D.D. Koeberl, Enhanced response to enzyme replacement therapy in Pompe disease after the induction of immune tolerance, Am. J. Hum. Genet. 81 (2007) 1042–1049.
[167] R.M. Boustany, Lysosomal storage diseases–the horizon expands, Nat. Rev. Neurol. 9 (2013) 583–598.
[168] P.A. Doerfler, A.G. Todd, N. Clément, D.J. Falk, S. Nayak, R.W. Herzog, B.J. Byrne, Copackaged AAV9 vectors promote simultaneous immune tolerance and phenotypic correction of pompe disease, Hum. Gene Ther. 27 (2016) 43–59.