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Immune responses in the mammalian inner ear and their implications for AAV-mediated inner ear gene therapy

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ABSTRACT

Adeno-associated virus (AAV)-mediated inner ear gene therapy is a promising treatment option for hearing loss and dizziness. Several studies have shown that AAV-mediated inner ear gene therapy can be applied to various mouse models of hereditary hearing loss to improve their auditory function. Despite the increase in AAV-based animal and clinical studies aiming to rescue auditory and vestibular functions, little is currently known about the host immune responses to AAV in the mammalian inner ear. It has been reported that the host immune response plays an important role in the safety and efficacy of viral-mediated gene therapy. Therefore, in order for AAV-mediated gene therapy to be successfully and safely translated into patients with hearing loss and dizziness, a better understanding of the host immune responses to AAV in the inner ear is critical. In this review, we summarize the current knowledge on host immune responses to AAV-mediated gene therapy in the mammalian inner ear and other organ systems. We also outline the areas of research that are critical for ensuring the safety and efficacy of AAV-mediated inner ear gene therapy in future clinical and translational studies.

1. Introduction

Hearing loss is the most common sensory deficit worldwide. According to World Health Organization, over 460 million people are affected by hearing loss, and this prevalence is expected to continue to rise due to an aging population (https://www.who.int/news-room/fact-sheets/detail/deafness-and-hearing-loss). Hearing loss can be caused by dysfunction or damage to a wide variety of highly specialized cell types in the cochlea due to genetic or environmental factors. Since most cell types in the mature mammalian cochlea are incapable of regeneration, the treatment options for patients with hearing loss are restricted to hearing aids, auditory rehabilitation, and cochlear implants. While these interventions provide benefits for some individuals, they still fail to address the underlying pathology of hearing loss and do not restore natural hearing. Hence, recent research has been focused on developing novel therapeutic strategies to protect or repair hair cells, auditory neurons, and other epithelial cell types of the inner ear.

Inner ear gene therapy is a promising treatment for hearing loss. It involves the delivery of genetic materials into the inner ear, which may restore the auditory function or protect against inner ear damage (Chien et al., 2015). The success of gene therapy depends in part on the viral vector and its ability to deliver genetic materials to the targeted cells efficiently (Chien et al., 2015). Currently, adeno-associated virus (AAV) is amongst the most frequently used viral vectors in human gene therapy clinical trials.

AAV is a non-enveloped virus with a single-stranded DNA genome. It belongs to the family of *Parvoviridae* (Fig. 1) (Naso et al., 2017). It is a commonly used viral vector for gene therapy applications because of its advantageous features, such as high transduction efficiency and the ability to engineer the viral capsid to target different cell types (Chien et al., 2015). Furthermore, AAV

Abbreviations: AAV, Adeno-associated virus; PAMPs, pathogen-associated molecular patterns; PRRs, pattern-recognition receptors; TLRs, Toll-like receptors; APC, Antigen Presenting Cell.

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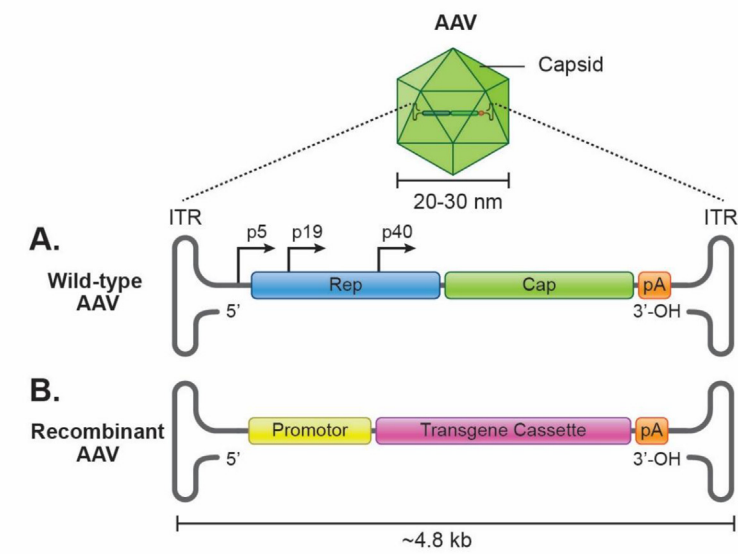

Fig. 1. Schematics of the AAV genome organization. (A) Wild-type AAV genome is comprised of two sets of open reading frames (ORFs): *Rep* (replication) and *Cap* (capsid), that encode viral genes along with the polyadenylation site (poly(A) tail; labeled "pA") flanked by two inverted terminal repeats (ITRs). *Rep* ORF encodes four non-structural proteins that regulate AAV replication and packaging of the viral ssDNA genome into pre-formed capsids. *Cap* ORF encodes three structural proteins that assemble into capsid shell and assembly-activating protein responsible for the viral structure. The p5 and p19 promoters regulate *Rep* gene expression and the p40 promoter regulates *Cap* gene expression. (B) Recombinant AAV used for gene therapy contains a heterologous promoter that drives the expression of the transgene and a transgene expression cassette inserted between the ITRs that replace the *Rep* and *Cap* genes.

is replication-deficient and nonpathogenic in humans. It can also achieve stable, long-term gene expression in animal models and humans (Chien et al., 2015).

Despite the advantageous features of AAV as a gene therapy viral vector, it has been shown that AAV can trigger immune responses in the host organisms (Amado et al., 2010; Manno et al., 2006; Mingozzi and Buning, 2015). The immune system plays a critical role in protecting the host against foreign pathogens, such as viruses. Despite its protective effects on the host, it has been shown that the host immune responses may pose a significant challenge to the efficacy and safety of viral gene therapy (Manno et al., 2006; Mingozzi and High, 2013). One example of this was reported in a clinical trial utilizing AAV-mediated gene therapy as a treatment for Duchenne muscular dystrophy, sponsored by Solid Biosciences (NCT03368742) (Duan, 2018). Patients who received AAV9-micro-dystrophin therapy presented with a decrease in platelet and red blood cell counts, and developed renal dysfunction only a few days after gene therapy administration. In addition, complement activation was also observed in these patients, suggesting the activation of innate immune response (Duan, 2018). In another clinical trial utilizing AAV-mediated therapy as a treatment for X-linked myotubular myopathy, sponsored by Astellas Pharma (NCT03199469), several patients who received high-dose AAV gene therapy died from liver failure, likely as a result of the activation of host immune responses (Wilson and Flotte, 2020). These examples highlight the critical role that immune response plays in the safety and success of viral-mediated gene therapy.

Several recent studies have shown that AAV-mediated gene therapy can be successfully applied to the mammalian cochlea to treat hearing loss in animal models (Askew and Chien, 2020a). However, it is still unclear whether AAV triggers any immune response in the mammalian inner ear. To maximize the therapeutic potential of gene therapy to preserve or restore hearing, it is important to understand the effects of host immune responses to viral vectors in the inner ear. In this review, we summarize our current understanding of AAV-mediated immune responses in the mammalian inner ear.

2. Overview of the immune response to pathogens

The immune system plays a critical role in defending against pathogens. The immune response can be classified into two main categories: innate and adaptive immunity, and each consists of cell-mediated and humoral immune responses (Parham, 2021).

2.1. Innate immunity

The innate immune response is the first line of defense to fight against pathogens. It is fast-acting and non-specific compared to the adaptive immune response. The cellular component is mediated by a subset of leukocytes such as neutrophils, eosinophils, mast cells, macrophages, monocytes, dendritic cells, and natural killer cells (NK cells) (Chaplin, 2010). Most of these leukocytes circulate in the vascular and lymphatic systems, and some leukocytes (such as tissue resident macrophages and dendritic cells) reside in various organ systems and help monitor the local environment.

The innate immune cells express pattern-recognition receptors (PRRs) on their cellular surfaces to detect the presence of pathogens and distinguish "self" from "non-self" (Kaur and Secord, 2021). PRRs recognize pathogen-associated molecular patterns (PAMPs), which are conserved molecular structures of pathogens. Bacterial molecules such as lipopolysaccharide (LPS) (gram-negative bacteria) and peptidoglycans (gram-positive bacteria) are well-known examples of PAMPs. Viruses also contain several structurally diverse PAMPs, such as surface glycoproteins or viral genomes (Simmonds and Aiewsakun, 2018). PRRs also detect damage-associated molecular patterns (DAMPs), which are the endogenous danger molecules released by damaged or dying cells as a result of inflammation or infection. PRRs can be broadly divided into five distinct classes: retinoic acid-inducible gene I (RIG-I)-like receptors (RLRs), nucleotide-binding oligomerization domain-like receptors (NLRs), C-type lectin receptors (CLRs), absent in melanoma 2 (AIM2)-like receptor (ALRs), and Toll-like receptors (TLRs).

TLRs are the best-characterized class of PRRs that sense a wide variety of molecular patterns (e.g., PAMPs and DAMPs) and

initiate the innate immune response. TLRs are largely classified into two subgroups based on their cellular localization: cell surface TLRs (TLR1, TLR2, TLR4, TLR5, TLR6) and intracellular TLRs localized in the endosome (TLR3, TLR7, TLR8, TLR9) (Kawasaki and Kawai, 2014). TLR2 and TLR4 on the cell surface detect viral proteins in the extracellular milieu (Aoshi et al., 2011; Lester and Li, 2014). The intracellular TLRs, TLR3, TLR7/8, and TLR9, recognize virus-derived double-stranded RNA (dsRNA), single-stranded RNA (ssRNA), and viral DNA that is rich in unmethylated cytosine-guanine dinucleotide (CpG)-DNA motif, respectively (Aoshi et al., 2011; Kano et al., 2022; Kawasaki and Kawai, 2014; Parham, 2021). Typically, activation of TLRs by PAMPs or DAMPs is commonly mediated by adaptor molecules such as Toll/interleukin-1 receptor domain-containing adapter inducing interferon-β (TRIF) or myeloid differentiation primary response 88 (MyD88), which activates the NF-κB (Wang et al., 2014). NF-κB activation leads to the secretion of inflammatory cytokines and chemokines, such as type I interferons (IFN-α and IFN-β), tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), interleukin-12 (IL-12), C-X-C motif chemokine ligand 8 (CXCL8), and C-C motif chemokine ligand 2 (CCL2). These cytokines and chemokines are essential for facilitating the recruitment of innate immune cells into the infected tissues, resulting in the clearance or inactivation of the PAMPs (Aoshi et al., 2011; Ivashkiv and Donlin, 2014; Poeck et al., 2010). Specifically, type I interferons are widely expressed during viral infection to activate an antiviral state in both infected and neighboring uninfected cells to prevent the virus from spreading and promote rapid viral clearance (Ivashkiv and Donlin, 2014; Schreiber, 2020).

Leukocytes involved in cell-mediated innate immunity utilize several other mechanisms to initiate protection against invading pathogens. Phagocytosis is a key mechanism used by neutrophils, macrophages, and dendritic cells for the engulfment and elimination of pathogens. Engulfed pathogens are delivered to an intracellular organelle called the phagosome that further fuse with lysosomes, an acidic membrane-bound organelle that contains degradative enzymes (e.g., proteases, lysozymes, or lipases), to form phagolysosomes (Lee et al., 2020; Uribe-Querol and Rosales, 2017). Macrophages and dendritic cells also serve as antigen-presenting cells (APCs). Following phagocytosis of the pathogen, APCs break down antigens into small amino acid fragments and present antigens on their cell surface for recognition by T cells to bridge the innate and adaptive immune responses (Roche and Furuta, 2015).

The humoral component of innate immunity is mediated largely through the complement system (Shishido et al., 2012). The complement system is comprised of a large group of soluble plasma proteins (>40 proteins) that are responsible for recognizing and clearing the invading pathogens. The system also participates in opsonization, a process by which a pathogen is coated with antibodies and targeted for elimination by phagocytes (Schartz and Tenner, 2020). The complement proteins are primarily produced by the liver and circulate in the blood in their inactive forms. Recognition of pathogens leads to the activation of proteolytic enzymes to cleave complement proteins, rendering them active. The complement system can be activated by three distinct pathways: classical, alternative, and lectin pathways (Thau et al., 2022). Activation of the complement system *via* one of these pathways leads to increased opsonization and phagocytosis activity by macrophages, neutrophils, and dendritic cells (Calcedo and Wilson, 2013; Schartz and Tenner, 2020; Thau et al., 2022).

2.2. Adaptive immunity

Adaptive immunity is initiated when the innate immune response is unable to completely eliminate pathogens. Similar to the innate immune system, the adaptive immune system also has both cellular and humoral components. Cell-mediated adaptive immunity involves the activation of naïve T cells through a process called "antigen presentation" that allows T cells to detect antigen molecules displayed on the surface of APCs, including B cells, macrophages, and dendritic cells. Antigen presentation is an integral part in the transition between innate and cell-mediated adaptive immunity. APCs phagocytose pathogens and degrade them into short amino acid peptides (8–25 aa), and bind peptide fragments to the major histocompatibility complex (MHC) molecules which are then translocated to the cell surface for antigen presentation (Chaplin, 2010). The MHC molecules are classified into two major classes, MHC class I and MHC class II. MHC class I molecules are expressed by nearly all nucleated cells, and they help to present endogenous antigens to cytotoxic CD8+ T cells that are critical for the rapid clearance of pathogens and pathogen-infected cells. Cytotoxic CD8+ T cells mediate their effector functions through the production of cytokines, such as TNF-α and IFN-γ, or by the release of cytotoxic granules, such as perforin or granzymes, to initiate apoptosis of infected cells (Demers et al., 2013; Kumar et al., 2017; Rosendahl Huber et al., 2014). MHC class II molecules are expressed by APCs and present exogenous antigens to helper CD4+ T cells that release cytokines to activate cytotoxic CD8+ T cells or B cells for antibody production (Parham, 2021). While there are several subsets characterized, the two major subsets of helper CD4+ T cells are Th1 and Th2 cells classified based on their profile of cytokine production. The Th1 cells secrete cytokines such as IFN-ɣ and TNF-α, which helps to activate macrophages and dendritic cells for cell-mediated immunity (Chaplin, 2010). The Th2 cells mediate the activation of humoral adaptive immunity, which is also called antibody-mediated immunity. They secrete cytokines, such as IL-4, IL-5, and IL-13, to stimulate naïve B cells to differentiate into plasma cells and to produce antibodies (typically IgM) specific for the antigen, or become memory B cells (Chaplin, 2010). Cytokines secreted by helper CD4+ T cells can stimulate B cells to undergo immunoglobulin heavy-chain class switching from IgM to IgG, IgA, or IgE. This process is called class switch recombination. Antibody-mediated neutralization of an invading pathogen can exert antiviral activity by interfering with interactions between virus and host cells or complement-mediated opsonization and cell lysis. However, antibody-mediated immunity is limited to the recognition of extracellular antigens, for example, on viruses circulating in the blood. Additionally, memory B cells circulate in the bloodstream in a quiescent state and, upon re-encounter with antigen, can be quickly activated to differentiate into antibody-secreting plasma cells, typically producing IgG.

3. Anatomy and immunity of the mammalian inner ear

The peripheral auditory system is comprised of the outer, middle, and inner ear. The outer and middle ear collects, amplifies, and transmits sound stimuli into the inner ear. The inner ear is enclosed by the otic capsule. The cochlea is the auditory organ in the inner ear. It is composed of three fluid-filled compartments called scala vestibuli, scala media, and scala tympani. The scala vestibuli and scala tympani are filled with perilymph, which has a low concentration of potassium ions, while the scala media is filled with endolymph, which contains a high concentration of potassium ions (Zdebik et al., 2009).

The sensory hair cells, which detect and process auditory stimuli, are located in the organ of Corti of the cochlea. Once sound enters the cochlea, the sensory hair cells convert the mechanical sound stimuli into neural signals and transmit them through the auditory nerve fibers of the spiral ganglion neurons into the auditory cortex in the brain (Hudspeth, 1989). In addition to cochlear hair cells, the stria vascularis is critical for normal hearing. The stria vascularis is a highly vascularized structure located in the

lateral wall of the scala media, and its main role is to generate endocochlear potential by actively pumping out ions against the concentration gradient into the endolymph and maintaining the unusually high concentration of the potassium ions in the scala media (Smith et al., 1954). This generates a positive potential of ~+80mV in the endolymph compared to the perilymph, in which this electrochemical gradient becomes the driving force for proper hair cell mechanotransduction (Nin et al., 2008).

The inner ear was initially thought to be immuno-privileged due to the fact that it is anatomically secluded from the external environment, and the presence of the blood-labyrinth barrier helps to tightly regulate the microenvironment in the inner ear from the blood circulation (Okano, 2014). However, subsequent studies have shown that the inner ear has an active immune system that helps to protect it against damage. One line of evidence that supports the presence of an active immune system in the inner ear is the presence of leukocytes (Hu et al., 2018). The most abundant leukocyte in the inner ear is macrophage, which account for ~80-85% of immune cells in the neonatal period, and ~95% of immune cells in adults (Hirose et al., 2005; Matern et al., 2017; Okano et al., 2008).

The macrophages are the major mediators of the immune response in the cochlea (Hu et al., 2018). They express markers such as CX3CR1, Iba1, F4/80, and CD68. The majority of them are located in the cochlear lateral wall (stria vascularis and spiral ligament), as well as near the spiral ganglion neurons in the modiolus and the Rosenthal's canal (Fig. 2A–D) (Hirose et al., 2005; Okano et al., 2008). A unique subset of macrophages, called the perivascular macrophages, are found in the stria vascularis (Ito et al., 2022; Zhang et al., 2012). They are closely associated with the blood vessels and regulate the blood-labyrinth barrier permeability that restricts the entry of substances from the blood into the inner ear, thereby maintaining the microenvironment of the inner ear.

Under normal physiological conditions, the organ of Corti is devoid of leukocytes, and the macrophages are located on the scala tympani side of the basilar membrane (Fig. 2D) (Cai et al., 2014; Hirose et al., 2005; Okano et al., 2008). In the modiolus and Rosenthal's canal, macrophages have been found to surround the spiral ganglion neurons and their nerve fibers (Okano et al., 2008), and are thought to play a role in maintaining synaptic homeostasis (Hu et al., 2018). In the presence of cochlear insult, these macrophages are activated and undergo dynamic changes to mediate cellular immunity (Frye et al., 2017; Yang et al., 2015). The acute immune responses in the mammalian cochlea have been examined under various conditions, including noise trauma, cochlear implantation, ototoxicity, and infection (Fujioka et al., 2006; Janesick et al., 2022; Koo et al., 2015; Matsunaga et al., 2020; Milon et al., 2021; Sung et al., 2019; Wakabayashi et al., 2010). Under these conditions, activated macrophages have been shown to clear damaged spiral ganglion neurons or hair cells *via* phagocytosis (Cai et al., 2014; Hirose et al., 2017). Macrophages have also been shown to promote the repair of damaged synapses after moderate noise exposure that leaves auditory hair cells intact but results in loss of hair cell synapses (Kaur et al., 2019; Manickam et al., 2023). Moreover, upon noise trauma or infection, a large influx of infiltrating leukocytes is found in the inner ear in addition to the cochlear resident macrophages. It has been shown that monocytes/macrophages account for the majority of the infiltrating inflammatory cells into the cochlea (Hirose et al., 2005; Rai et al., 2020; Yang et al., 2015).

In addition to the cochlea, the endolymphatic sac may also play a role in the immune response in the mammalian inner ear (Kampfe Nordstrom et al., 2018). The endolymphatic sac is a pouch-like structure that is connected to the inner ear by the endolymphatic duct. It plays an important role in ion transport and fluid absorption, and helps to maintain endolymphatic homeostasis (Honda et al., 2017; Honda et al., 2021). Several studies have

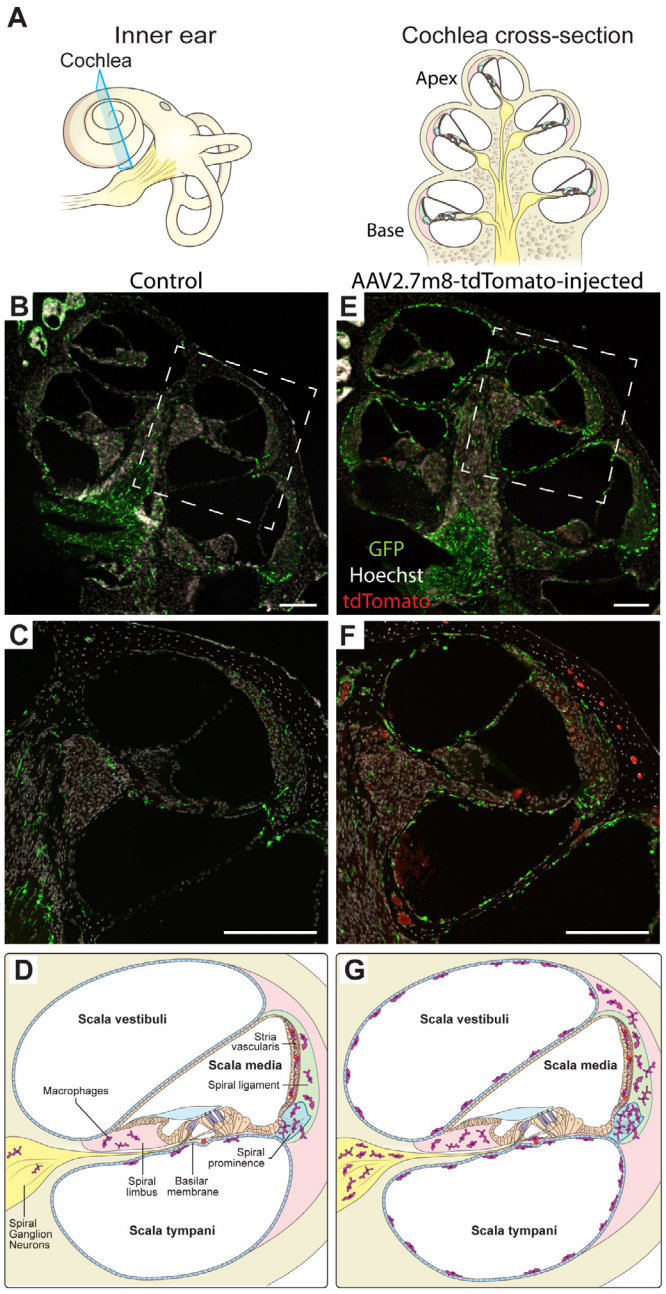

Fig. 2. AAV injection into the inner ear results in an increased number of macrophages in the cochlea. (A) Schematic diagrams of the inner ear and cochlear cross-section. (B and C) Confocal images showing the localization of macrophages in the cochlea harvested from an adult non-injected CX3CR1$^{GFP/GFP}$ mouse (age 9 weeks). The macrophages in the CX3CR1$^{GFP/GFP}$ mouse express GFP under the regulation of the promoter for the fractalkine receptor CX3CR1. Under normal conditions, resident macrophages are most abundantly located in the cochlear lateral wall and around the spiral ganglion neurons in the modiolus and the Rosenthal's canal. (E and F) Confocal images of cochlear sections from an adult CX3CR1$^{GFP/GFP}$ mouse (age 13 weeks) 14 days after AAV2.7m8-tdTomato (2.0×10^{10} GC) administration through the posterior semicircular canal. Increased number of macrophages were observed in the lateral wall, the modiolus, and the basilar membrane. Additionally, macrophages were observed along the lining of the perilymphatic space in all cochlear turns. (C and F) Magnified inset images from white dotted box in panels B and E. (D and G) Schematic representation of macrophage distribution in the middle cochlear turn as shown in panels **C** and **F**. (B,C,E,F) Scale bars represent 100 μm.

reported the presence of leukocytes such as macrophages in the endolymphatic sac (Bui et al., 1989; Kampfe Nordstrom et al., 2018; Okano et al., 2010; Rask-Andersen and Stahle, 1979, 1980). In addition, gene expression analysis of the human endolymphatic sac

from vestibular schwannoma patients showed the expression of several genes involved with immune responses, such as TLR4, TLR7, lactoferrin (iron-binding protein with antimicrobial activity), and β-defensin (small cationic antimicrobial peptides) (Moller et al., 2015). These data suggest that the mammalian inner ear has an active immune system and is capable of mounting robust immune responses against pathogens.

4. Adeno-associated virus (AAV) and AAV-mediated immune responses

4.1. Overview of AAV biology

AAV is a helper-dependent parvovirus with a linear single-stranded DNA genome of ~4.8 kb. Twelve AAV serotypes (AAV1 to AAV12) have been isolated from humans and non-human primates, with AAV2 being the most prevalent serotype (Wu et al., 2006). Different AAV serotypes have different tissue/cellular tropisms due to their binding of distinct host cell surface receptors. Therefore, they can infect a highly diverse range of dividing and non-dividing cell types (Summerford et al., 2016; Zincarelli et al., 2008). The genome of wild-type AAV is comprised of two open reading frames and viral genes (*Rep* and *Cap*) flanked by two inverted terminal repeats (ITRs) (Fig. 1A) (Earley et al., 2020; Penaud-Budloo et al., 2018; Yan et al., 2005). Recombinant AAV vectors, derived from wild-type AAV, have been genetically engineered to lack viral genes except for the ITRs that allow for the replication of the complementary DNA strand and facilitate the packaging of the exogenous genome of interest (Fig. 1B) (Wang et al., 2019). AAV genomes are primarily present as episomes or concatemers in the cell nucleus. However, during latency, the AAV genome can integrate into a specific genome locus known as AAVS1 (adeno-associated virus integration site 1; chromosome 19q13.4 qtr) in human cells, albeit at very low frequencies (0.1% occurrence) (Kotin et al., 1991, 1990; Linden et al., 1996a, 1996b; Samulski, 1993). This is primarily due to the sequence similarity (86%) between AAV ITR and host chromosome leading to *Rep* activity (Linden et al., 1996b; Meneses et al., 2000; Weitzman et al., 1994). Deletion of *Rep* gene in the AAV viral vectors designed for gene delivery significantly reduces the risk of AAV genome integration into the host chromosome, thereby avoiding the risk of insertional mutagenesis (Wang et al., 2019).

4.2. AAV-mediated immune responses in the mammalian inner ear

Innate immune responses against AAVs in animal models showed TLR9 involvement, followed by activation of MyD88, which increases the secretion of type I interferons and pro-inflammatory cytokines through the NF-κB signaling pathway (Martino and Markusic, 2020). Recombinant AAVs can also trigger the release of cytokines such as TNF-α, IFN-γ, CCL2, inducible protein-10, and type I interferons (IFN-α and IFN-β) (Nathwani et al., 2006). Primary human non-parenchymal liver cells transduced with AAV *in vitro* showed activation of NK-κB through TLR2, followed by up-regulation of pro-inflammatory cytokines (Hosel et al., 2012). In an *in vivo* study, IL-6 and CCL2 were increased in the liver cells within 6 hours after systemic AAV8 delivery (Suzuki et al., 2013). Following AAV-mediated gene delivery to the host cells, components of the AAV vector, including capsid, promoter, transgene, poly(A) tail, and ITR can be processed and presented as antigens on MHC molecules for antigen presentation to T cells (Li and Samulski, 2020).

Adaptive immune responses against AAVs are primarily mediated by TLR9-MyD88 pathway to activate NF-κB and induce cytokine production in helper CD4+ T cells that in turn activate cytotoxic CD8+ T cells. The helper CD4+ T cells also activate B cells for antibody production (Rogers et al., 2015; Sudres et al., 2012). Furthermore, long-lasting immunity (also known as pre-existing immunity) against AAV is mediated by memory B cells, memory T cells, and pre-existing antibodies. Specifically, clinical studies in healthy children have shown that natural exposure to wild-type AAV is, in part, responsible for pre-existing immunity (Calcedo et al., 2011; Erles et al., 1999; Mingozzi and High, 2013). In addition, patients that have received AAV-mediated gene therapy for the first time can also develop humoral and cell-mediated immunity against AAV. This newly developed immunity to AAV may be triggered by additional administration of AAV-mediated gene therapy at a later date. Approximately 70% of the adult population was found to have IgG antibodies against AAV1 and AAV2, and 40–50% has antibodies against AAV5, AAV6, AAV8, and AAV9 (Boutin et al., 2010; Calcedo et al., 2009). Neutralizing antibodies against AAVs have also been found in 15–20% of the younger populations (between one year old to adolescence) (Calcedo et al., 2011). The pre-existing immunity to AAV can significantly affect the transduction efficiency and potency of AAV-mediated gene therapy (Fitzpatrick et al., 2018; Hosel et al., 2012; Mingozzi et al., 2013b; Mingozzi and High, 2013; Vandamme et al., 2017).

AAV is one of the most commonly used viral vectors for human gene therapy clinical trials due to the low immunogenicity, safety profile, and high transduction efficiency (Steines et al., 2016; Vidovic et al., 2016). AAV has also become the primary choice of inner ear gene therapy studies and has been shown to mediate efficient transduction in a wide variety of cell types in the vestibular organs (Tan et al., 2019; Wang et al., 2014) and in the cochlea, including the organ of Corti, spiral ganglion neurons, spiral ligaments, stria vascularis, and spiral limbus (Akil et al., 2019; Hu et al., 2019; Isgrig et al., 2022, 2019; Ivanchenko et al., 2021; Landegger et al., 2017; Lee et al., 2020; Tan et al., 2019). Numerous studies have shown that AAV-mediated gene replacement therapy can successfully improve the auditory function in several animal models of hereditary hearing loss (Askew and Chien, 2020b). These studies have used local AAV gene delivery directly into the inner ear (e.g., round window or semicircular canal delivery), which has been shown to result in high transduction efficiencies. In addition, wild-type and heterozygous littermates that were injected with the same AAV vectors showed minimal change in hearing thresholds, suggesting AAVs as excellent viral vectors for safe therapeutic gene delivery to the mammalian inner ear. Unfortunately, most of these studies did not specifically examine the host immune responses in the inner ear after AAV-mediated gene delivery. Therefore, more research in this area is greatly needed.

Despite the lack of studies that have directly examined the host immune responses in the mammalian inner ear after AAV-mediated gene delivery, a few studies have indirectly tackled this important topic. In a study by Landegger et al. (2017), where the synthetic AAV Anc80L65 was injected into the neonatal mouse inner ear *via* the round window, they found robust transduction of the inner and outer hair cells in the cochlea. They also reported that low levels of neutralizing antibodies were detected in the serum of injected mice (Landegger et al., 2017). In another study, Isgrig et al. (2019) compared two synthetic AAVs, AAV2.7m8 and AAV8BP2, administered in the neonatal mouse inner ear *via* the posterior semicircular canal (Isgrig et al., 2019). The authors showed that AAV2.7m8 (9.75×10^9 GC) was capable of transducing cochlear inner hair cells, outer hair cells, inner pillar cells, and inner phalangeal cells with high efficiency and with no significant change in hearing, as assessed by auditory brainstem response (ABR). In contrast, AAV8BP2 (1.10×10^{10} GC) showed only ~50% transduction efficiency in inner hair cells and outer hair cells. Mice injected with AAV8BP2 had elevated ABR thresholds compared to control mice. Examination of the cochlea in these animals showed evidence of immune cell infiltration, suggesting

activation of innate immunity. When AAV8BP2 was administered at half dose (5.5×10^9 GC), ABR thresholds were comparable to those of control mice, but the transduction efficiency also decreased (Isgrig et al., 2019). Recent data from our group showed that injection of AAV2.7m8-tdTomato (2.0×10^{10} GC) into the inner ear *via* the posterior semicircular canal increased macrophage accumulation in the cochlear lateral wall, the modiolus, and the basilar membrane. Macrophages were also found along the lining of the perilymphatic space in all three cochlear turns (base, middle, apex), while macrophages were minimally detected in this region from the cochleae of non-injected control mice (Fig. 2). Additionally, studies in non-human primate showed that round window administration of AAV-S-EGFP at a low dose (8×10^{10} GC) had low-to-moderate transduction efficiency in hair cells, while higher doses (5.8×10^{11} GC; 4.7×10^{11} GC) nearly transduced 100% of hair cells throughout all regions in the cochlea (Ivanchenko et al., 2021). Animals administered with high dose AAV-S-CBA-GFP (4.7×10^{11} GC) showed normal tissue morphology and structure, but immune cell infiltration was observed in the modiolus, though this did not affect AAV-mediated gene transfer. In summary, administration of AAV into the inner ear showed high transduction efficiency and minimal immunogenicity, as demonstrated in various animal studies (Chien et al., 2015; Landegger et al., 2017; Liu et al., 2005; Verdoodt et al., 2021; Wang et al., 2019). However, the assessment of immunogenicity has been based on rather crude histology, such as hematoxylin & eosin (H&E) staining to examine inflammatory cell infiltration into the inner ear. Given that inflammatory responses can be cytotoxic at the cellular level, while maintaining normal tissue morphology and structure, it is important to employ more sophisticated assays which will allow for the assessment of the various immune cell types and antibody responses against AAV-mediated gene therapy in the inner ear.

4.3. Immune responses in AAV-mediated gene therapy clinical trials

The first human clinical trial of AAV-mediated gene therapy occurred in 1996, where AAV2 was used to deliver cystic fibrosis transmembrane regulator (CFTR) cDNA to patients with cystic fibrosis, a genetic disorder caused by mutations in the *CFTR* gene leading to severe damage primarily in the lungs and the digestive system (Flotte et al., 1996). The study was approved by the FDA following multiple animal model studies which achieved long-term gene transfer without developing adverse immune responses (Fisher et al., 1997; Kessler et al., 1996; Xiao et al., 1996). In contrast, adverse effects of the host immune responses to AAV were reported in a clinical trial for hemophilia B, a hereditary bleeding disorder caused by mutations in the gene for coagulation factor IX (FIX). In this clinical trial, AAV2 was used to deliver FIX cDNA to hemophilia B patients (Manno et al., 2003, 2006). Although AAV2 was able to achieve transduction in human hepatocytes without long-term toxicity for many patients, a decline in FIX levels occurred over an 8-week period. In addition, the destruction of transduced hepatocytes occurred in these patients through CD8+ T cell-mediated cytotoxicity, limiting long-term expression of the transgene (Manno et al., 2006; Sabatino et al., 2005; Yamashita et al., 2018). These cytotoxic CD8+ T cells are capable of targeting the capsid components that are conserved throughout many AAV serotypes or can become memory T cells to prevent future infections (Hui et al., 2015). In addition to the AAV capsid, the host immune system can also target the transgene delivered by AAV. For example, it has been reported that patients with Duchenne muscular dystrophy (an X-linked genetic disorder caused by mutations in the dystrophin gene which leads to progressive muscle wasting) who received AAV2-mediated gene therapy to deliver a mini-dystrophin transgene developed dystrophin-specific T cell immunity (Mendell et al., 2010).

To overcome pre-existing and newly developed humoral and cellular immunity, various approaches have been investigated in animal models and pre-clinical studies (Ertl and High, 2017; Martino and Markusic, 2020), such as switching AAV serotypes (Majowicz et al., 2017), altering AAV capsids (Mingozzi and High, 2013), administration of immunosuppressants (Mingozzi et al., 2012), or administration of empty viral capsids to absorb neutralizing antibodies (Mingozzi et al., 2013a). An example of this can be found in clinical trials of patients with Leber congenital amaurosis (LCA), a rare form of inherited retinal dystrophy causing severe visual impairment early in life. LCA is caused by mutations in more than two dozen genes, including a gene that encodes retinal pigment epithelium-specific 65 kDa protein (RPE65). LCA patients who received AAV2-RPE65 gene therapy also received a 5-week course of prednisolone given orally starting 1 week prior to gene therapy delivery to suppress inflammation (Bainbridge et al., 2008). These patients displayed only a mild postoperative intraocular inflammation, and no significant adverse events were reported. Long-term follow up of these patients showed chronic intraocular inflammation in 5 out of 8 patients who received a higher dose of the gene therapy, but no intraocular inflammation was found in any patients who received a lower dose of the gene therapy (Bainbridge et al., 2008). Some patients developed neutralizing antibodies against AAV2 and/or AAV2-reactive T cells, but no immune responses were detected against the RPE65 transgene product. This therapy was approved by the FDA as a treatment for LCA (Luxturna).

Another AAV-based gene therapy recently approved by FDA is Zolgensma for spinal muscular atrophy. Spinal muscular atrophy is a devastating autosomal recessive disorder caused by mutations in the survival motor neuron 1 (SMN1) gene (Burghes and Beattie, 2009; Monani et al., 1999). Patients with type 1 spinal muscular atrophy have the most severe disease phenotype and present with progressive muscle weakness before 6 months of age. By two years of age, many patients have difficulty speaking, swallowing, and breathing, which may require permanent ventilation support (Burghes and Beattie, 2009; Monani et al., 1999). Following successful pre-clinical studies with AAV9-mediated SMN1 gene replacement (Dominguez et al., 2011; Foust et al., 2010), a clinical trial of type 1 spinal muscular atrophy was approved to move forward (NCT02122952). Initially, no immunosuppression was used in patients enrolled in this study. However, soon after gene therapy delivery, the liver enzyme alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were elevated, indicating possible hepatic inflammation. Upon this finding, prednisolone was incorporated into the clinical protocol to suppress the immune response, which decreased the incidence of ALT/AST elevation in subsequent study subjects. In addition, the viral titer administered was also reduced to decrease immune activation (Mendell et al., 2017). In 2019, onasemnogene abeparvovec (Zolgensma) was approved by the FDA for the treatment of type 1 spinal muscular atrophy. Recently, there have been two cases of deaths in children who received Zolgensma due to liver failure (Philippidis, 2022). This highlights the importance of studying the immune responses of viral-mediated gene therapy to ensure patient safety.

5. Conclusions

Inner ear gene therapy is a rapidly evolving field. Over the past decade, several studies have shown that AAV-mediated inner ear gene therapy is effective at improving auditory function in various small animal models of hearing loss. However, little attention has been paid to the immune responses triggered by AAV-mediated gene therapy in the inner ear. In this review, we provided an overview of AAV-mediated immune responses in the setting of gene therapy delivery in the mammalian inner ear. It is clear that

more studies on the immune activation in the mammalian inner ear in response to AAV-mediated gene therapy are needed in order to ensure the safety of future inner ear gene therapy clinical trials. Many important questions remain to be answered, such as:

- Are there differences in AAV-mediated immune responses in the inner ear among different animal species?
- Is the degree of inflammation dependent on viral dose, serotype, route of administration, or repeated injections of viral vector upon local delivery into the inner ear?

Additionally, many people have been exposed to AAVs in the past and have generated neutralizing antibodies against them. As pre-existing immunity interferes with viral transduction efficiency and facilitates immune activation, this raises the following important questions:

- Does pre-existing immunity affect the efficacy and safety of inner ear gene therapy?
- Can the use of immunosuppressive drugs (e.g., corticosteroids or rapamycin) that transiently suppress viral-triggered immune responses increase the efficacy and safety of AAV-mediated inner ear gene therapy?

Lastly, several mechanisms have been identified by which viral vectors can activate the immune responses in animal models as previously discussed. This raises the following key questions:

- Can the viral capsid be engineered to remove or modify the epitopes that are commonly recognized by the immune cells?
- Can components of the viral genome that may be recognized by the immune system be modified to circumvent immune activation?

Considerable progress has been made in the development of AAV-mediated gene delivery systems that resulted in FDA-approved products for clinical use in other organ systems (e.g. Luxturna and Zolgensma). However, few data exist on how the human inner ear will respond to AAV-mediated gene delivery. A better understanding of AAV-mediated immune responses is paramount to developing definitive AAV-mediated gene therapies that maximize safety and efficacy in the human inner ears. It is our hope that this review will inspire investigators to not only focus on the assessment of transduction efficiency and tropism of viral vectors, but also to investigate the mechanisms that induce diverse immune responses triggered by them.

Declaration of Competing Interest

The authors have no conflict of interest to declare.

Data availability

Data will be made available on request.

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References

Akil, O., Dyka, F., Calvet, C., Emptoz, A., Lahlou, G., Nouaille, S., Boutet de Monvel, J., Hardelin, J.P., Hauswirth, W.W., Avan, P., Petit, C., Safieddine, S., Lustig, L.R., 2019. Dual AAV-mediated gene therapy restores hearing in a DFNB9 mouse model. Proc. Natl. Acad. Sci. U. S. A. 116 (10), 4496–4501. doi:10.1073/pnas.1817537116.

Amado, D., Mingozzi, F., Hui, D., Bennicelli, J.L., Wei, Z., Chen, Y., Bote, E., Grant, R.L., Golden, J.A., Narfstrom, K., Syed, N.A., Orlin, S.E., High, K.A., Maguire, A.M., Bennett, J., 2010. Safety and efficacy of subretinal readministration of a viral vector in large animals to treat congenital blindness. Sci. Transl. Med. 2 (21), 21ra16. doi:10.1126/scitranslmed.3000659, 2/21/21ra16 [pii].

Aoshi, T., Koyama, S., Kobiyama, K., Akira, S., Ishii, K.J., 2011. Innate and adaptive immune responses to viral infection and vaccination. Curr. Opin. Virol. 1 (4), 226–232. doi:10.1016/j.coviro.2011.07.002.

Askew, C., Chien, W.W., 2020a. Adeno-associated virus gene replacement for recessive inner ear dysfunction: progress and challenges. Hear. Res., 107947 doi:10.1016/j.heares.2020.107947.

Askew, C., Chien, W.W., 2020b. Adeno-associated virus gene replacement for recessive inner ear dysfunction: progress and challenges. Hear. Res. 394, 107947. doi:10.1016/j.heares.2020.107947.

Bainbridge, J.W., Smith, A.J., Barker, S.S., Robbie, S., Henderson, R., Balaggan, K., Viswanathan, A., Holder, G.E., Stockman, A., Tyler, N., Petersen-Jones, S., Bhattacharya, S.S., Thrasher, A.J., Fitzke, F.W., Carter, B.J., Rubin, G.S., Moore, A.T., Ali, R.R., 2008. Effect of gene therapy on visual function in Leber's congenital amaurosis. N. Engl. J. Med. 358 (21), 2231–2239. doi:10.1056/NEJMoa0802268.

Boutin, S., Monteilhet, V., Veron, P., Leborgne, C., Benveniste, O., Montus, M.F., Masurier, C., 2010. Prevalence of serum IgG and neutralizing factors against adeno-associated virus (AAV) types 1, 2, 5, 6, 8, and 9 in the healthy population: implications for gene therapy using AAV vectors. Hum. Gene. Ther. 21 (6), 704–712. doi:10.1089/hum.2009.182.

Bui, H.T., Linthicum Jr., F.H., Hofman, F.M., Bowman, C.A., House, W.F., 1989. An immunohistochemical study of the endolymphatic sac in patients with acoustic tumors. Laryngoscope 99 (8 Pt 1), 775–778. doi:10.1288/00005537-198908000-00001.

Burghes, A.H., Beattie, C.E., 2009. Spinal muscular atrophy: why do low levels of survival motor neuron protein make motor neurons sick? Nat. Rev. Neurosci. 10 (8), 597–609. doi:10.1038/nrn2670.

Cai, Q., Vethanayagam, R.R., Yang, S., Bard, J., Jamison, J., Cartwright, D., Dong, Y., Hu, B.H., 2014. Molecular profile of cochlear immunity in the resident cells of the organ of Corti. J. Neuroinflamm. 11, 173. doi:10.1186/s12974-014-0173-8.

Calcedo, R., Morizono, H., Wang, L., McCarter, R., He, J., Jones, D., Batshaw, M.L., Wilson, J.M., 2011. Adeno-associated virus antibody profiles in newborns, children, and adolescents. Clin. Vaccine Immunol. 18 (9), 1586–1588. doi:10.1128/CVI.05107-11.

Calcedo, R., Vandenberghe, L.H., Gao, G., Lin, J., Wilson, J.M., 2009. Worldwide epidemiology of neutralizing antibodies to adeno-associated viruses. J. Infect. Dis. 199 (3), 381–390. doi:10.1086/595830.

Calcedo, R., Wilson, J.M., 2013. Humoral immune response to AAV. Front. Immunol. 4, 341. doi:10.3389/fimmu.2013.00341.

Chaplin, D.D., 2010. Overview of the immune response. J. Allergy Clin. Immunol. 125 (2 Suppl 2), S3–23. doi:10.1016/j.jaci.2009.12.980.

Chien, W.W., Monzack, E.L., McDougald, D.S., Cunningham, L.L., 2015. Gene therapy for sensorineural hearing loss. Ear Hear. 36 (1), 1–7. doi:10.1097/AUD.0000000000000088.

Demers, K.R., Reuter, M.A., Betts, M.R., 2013. CD8(+) T-cell effector function and transcriptional regulation during HIV pathogenesis. Immunol. Rev. 254 (1), 190–206. doi:10.1111/imr.12069.

Dominguez, E., Marais, T., Chatauret, N., Benkhelifa-Ziyyat, S., Duque, S., Ravassard, P., Carcenac, R., Astord, S., Pereira de Moura, A., Voit, T., Barkats, M., 2011. Intravenous scAAV9 delivery of a codon-optimized SMN1 sequence rescues SMA mice. Hum. Mol. Genet. 20 (4), 681–693. doi:10.1093/hmg/ddq514.

Duan, D., 2018. Systemic AAV micro-dystrophin gene therapy for duchenne muscular dystrophy. Mol. Ther. 26 (10), 2337–2356. doi:10.1016/j.ymthe.2018.07.011.

Earley, L.F., Conatser, L.M., Lue, V.M., Dobbins, A.L., Li, C., Hirsch, M.L., Samulski, R.J., 2020. Adeno-associated virus serotype-specific inverted terminal repeat sequence role in vector transgene expression. Hum. Gene. Ther. 31 (3-4), 151–162. doi:10.1089/hum.2019.274.

Erles, K., Sebokova, P., Schlehofer, J.R., 1999. Update on the prevalence of serum antibodies (IgG and IgM) to adeno-associated virus (AAV). J. Med. Virol. 59 (3), 406–411. doi: 10.1002/(sici)1096-9071(199911)59:3⟨406::aid-jmv22⟩3.0.co;2-n.

Ertl, H.C.J., High, K.A., 2017. Impact of AAV capsid-specific T-cell responses on design and outcome of clinical gene transfer trials with recombinant adeno-associated viral vectors: an evolving controversy. Hum. Gene Ther. 28 (4), 328–337. doi:10.1089/hum.2016.172.

Fisher, K.J., Jooss, K., Alston, J., Yang, Y., Haecker, S.E., High, K., Pathak, R., Raper, S.E., Wilson, J.M., 1997. Recombinant adeno-associated virus for muscle directed gene therapy. Nat. Med. 3 (3), 306–312. doi:10.1038/nm0397-306.

Fitzpatrick, Z., Leborgne, C., Barbon, E., Masat, E., Ronzitti, G., van Wittenberghe, L., Vignaud, A., Collaud, F., Charles, S., Simon Sola, M., Jouen, F., Boyer, O., Mingozzi, F., 2018. Influence of pre-existing anti-capsid neutralizing and binding antibodies on AAV vector transduction. Mol. Ther. Methods Clin. Dev. 9, 119–129. doi:10.1016/j.omtm.2018.02.003.

Flotte, T., Carter, B., Conrad, C., Guggino, W., Reynolds, T., Rosenstein, B., Taylor, G., Walden, S., Wetzel, R., 1996. A phase I study of an adeno-associated virus-CFTR gene vector in adult CF patients with mild lung disease. Hum. Gene. Ther. 7 (9), 1145–1159. doi:10.1089/hum.1996.7.9-1145.

Foust, K.D., Wang, X., McGovern, V.L., Braun, L., Bevan, A.K., Haidet, A.M., Le, T.T., Morales, P.R., Rich, M.M., Burghes, A.H., Kaspar, B.K., 2010. Rescue of the spinal muscular atrophy phenotype in a mouse model by early postnatal delivery of SMN. Nat. Biotechnol. 28 (3), 271–274. doi:10.1038/nbt.1610.

Frye, M.D., Yang, W., Zhang, C., Xiong, B., Hu, B.H., 2017. Dynamic activation of basilar membrane macrophages in response to chronic sensory cell degeneration in aging mouse cochleae. Hear. Res. 344, 125–134. doi:10.1016/j.heares.2016.11.003.

Fujioka, M., Kanzaki, S., Okano, H.J., Masuda, M., Ogawa, K., Okano, H., 2006. Proinflammatory cytokines expression in noise-induced damaged cochlea. J. Neurosci. Res. 83 (4), 575–583. doi:10.1002/jnr.20764.

Hirose, K., Discolo, C.M., Keasler, J.R., Ransohoff, R., 2005. Mononuclear phagocytes migrate into the murine cochlea after acoustic trauma. J. Compar. Neurol. 489 (2), 180–194. doi:10.1002/cne.20619.
Hirose, K., Rutherford, M.A., Warchol, M.E., 2017. Two cell populations participate in clearance of damaged hair cells from the sensory epithelia of the inner ear. Hear. Res. 352, 70–81. doi:10.1016/j.heares.2017.04.006.
Honda, K., Kim, S.H., Kelly, M.C., Burns, J.C., Constance, L., Li, X., Zhou, F., Hoa, M., Kelley, M.W., Wangemann, P., Morell, R.J., Griffith, A.J., 2017. Molecular architecture underlying fluid absorption by the developing inner ear. Elife 6. doi:10.7554/eLife.26851.
Honda, K., Lee, H.J., Griffith, A.J., Roux, I., 2021. Dissection of the endolymphatic sac from mice. J. Vis. Exp. (169) doi:10.3791/62375.
Hosel, M., Broxtermann, M., Janicki, H., Esser, K., Arzberger, S., Hartmann, P., Gillen, S., Kleeff, J., Stabenow, D., Odenthal, M., Knolle, P., Hallek, M., Protzer, U., Buning, H., 2012. Toll-like receptor 2-mediated innate immune response in human nonparenchymal liver cells toward adeno-associated viral vectors. Hepatology 55 (1), 287–297. doi:10.1002/hep.24625.
Hu, B.H., Zhang, C., Frye, M.D., 2018. Immune cells and non-immune cells with immune function in mammalian cochleae. Hear. Res. 362, 14–24. doi:10.1016/j.heares.2017.12.009.
Hu, X., Wang, J., Yao, X., Xiao, Q., Xue, Y., Wang, S., Shi, L., Shu, Y., Li, H., Yang, H., 2019. Screened AAV variants permit efficient transduction access to supporting cells and hair cells. Cell Discov. 5, 49. doi:10.1038/s41421-019-0115-9.
Hudspeth, A.J., 1989. How the ear's works work. Nature 341 (6241), 397–404. doi:10.1038/341397a0.
Hui, D.J., Edmonson, S.C., Podsakoff, G.M., Pien, G.C., Ivanciu, L., Camire, R.M., Ertl, H., Mingozzi, F., High, K.A., Basner-Tschakarjan, E., 2015. AAV capsid CD8+ T-cell epitopes are highly conserved across AAV serotypes. Mol. Ther. Methods Clin. Dev. 2, 15029. doi:10.1038/mtm.2015.29.
Isgrig, K., Ishibashi, Y., Lee, H.J., Zhu, J., Grati, M., Bennett, J., Griffith, A.J., Roux, I., Chien, W.W., 2022. AAV8BP2 and AAV8 transduce the mammalian cochlear lateral wall and endolymphatic sac with high efficiency. Mol. Ther. Methods Clin. Dev. 26, 371–383. doi:10.1016/j.omtm.2022.07.013.
Isgrig, K., McDougald, D.S., Zhu, J., Wang, H.J., Bennett, J., Chien, W.W., 2019. AAV2.7m8 is a powerful viral vector for inner ear gene therapy. Nat. Commun. 10 (1), 427. doi:10.1038/s41467-018-08243-1.
Ito, T., Kurata, N., Fukunaga, Y., 2022. Tissue-resident macrophages in the stria vascularis. Front. Neurol. 13, 818395. doi:10.3389/fneur.2022.818395.
Ivanchenko, M.V., Hanlon, K.S., Hathaway, D.M., Klein, A.J., Peters, C.W., Li, Y., Tamvakologos, P.I., Nammour, J., Maguire, C.A., Corey, D.P., 2021. AAV-S: a versatile capsid variant for transduction of mouse and primate inner ear. Mol. Ther. Methods Clin. Dev. 21, 382–398. doi:10.1016/j.omtm.2021.03.019.
Ivashkiv, L.B., Donlin, L.T., 2014. Regulation of type I interferon responses. Nat. Rev. Immunol. 14 (1), 36–49. doi:10.1038/nri3581.
Janesick, A.S., Scheibinger, M., Benkafadar, N., Kirti, S., Heller, S., 2022. Avian auditory hair cell regeneration is accompanied by JAK/STAT-dependent expression of immune-related genes in supporting cells. Development 149 (8). doi:10.1242/dev.200113.
Kampfe Nordstrom, C., Danckwardt-Lilliestrom, N., Laurell, G., Liu, W., Rask-Andersen, H., 2018. The human endolymphatic sac and inner ear immunity: macrophage interaction and molecular expression. Front. Immunol. 9, 3181. doi:10.3389/fimmu.2018.03181.
Kano, N., Ong, G.H., Ori, D., Kawai, T., 2022. Pathophysiological role of nucleic acid-sensing pattern recognition receptors in inflammatory diseases. Front. Cell Infect. Microbiol. 12, 910654. doi:10.3389/fcimb.2022.910654.
Kaur, B.P., Secord, E., 2021. Innate immunity. Immunol. Allergy Clin. North Am. 41 (4), 535–541. doi:10.1016/j.iac.2021.07.003.
Kaur, T., Clayman, A.C., Nash, A.J., Schrader, A.D., Warchol, M.E., Ohlemiller, K.K., 2019. Lack of fractalkine receptor on macrophages impairs spontaneous recovery of ribbon synapses after moderate noise trauma in C57BL/6 mice. Front. Neurosci. 13, 620. doi:10.3389/fnins.2019.00620.
Kawasaki, T., Kawai, T., 2014. Toll-like receptor signaling pathways. Front. Immunol. 5, 461. doi:10.3389/fimmu.2014.00461.
Kessler, P.D., Podsakoff, G.M., Chen, X., McQuiston, S.A., Colosi, P.C., Matelis, L.A., Kurtzman, G.J., Byrne, B.J., 1996. Gene delivery to skeletal muscle results in sustained expression and systemic delivery of a therapeutic protein. Proc. Natl. Acad. Sci. U. S. A. 93 (24), 14082–14087. doi:10.1073/pnas.93.24.14082.
Koo, J.W., Quintanilla-Dieck, L., Jiang, M., Liu, J., Urdang, Z.D., Allensworth, J.J., Cross, C.P., Li, H., Steyger, P.S., 2015. Endotoxemia-mediated inflammation potentiates aminoglycoside-induced ototoxicity. Sci. Transl. Med. 7 (298), 298ra118. doi:10.1126/scitranslmed.aac5546.
Kotin, R.M., Menninger, J.C., Ward, D.C., Berns, K.I., 1991. Mapping and direct visualization of a region-specific viral DNA integration site on chromosome 19q13-qter. Genomics 10 (3), 831–834. doi:10.1016/0888-7543(91)90470-y.
Kotin, R.M., Siniscalco, M., Samulski, R.J., Zhu, X.D., Hunter, L., Laughlin, C.A., McLaughlin, S., Muzyczka, N., Rocchi, M., Berns, K.I., 1990. Site-specific integration by adeno-associated virus. Proc. Natl. Acad. Sci. U. S. A. 87 (6), 2211–2215. doi:10.1073/pnas.87.6.2211.
Kumar, S.R.P., Hoffman, B.E., Terhorst, C., de Jong, Y.P., Herzog, R.W., 2017. 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. J. Am. Soc. Gene Ther. 25 (4), 880–891. doi:10.1016/j.ymthe.2017.02.014.
Landegger, L.D., Pan, B., Askew, C., Wassmer, S.J., Gluck, S.D., Galvin, A., Taylor, R., Forge, A., Stankovic, K.M., Holt, J.R., Vandenberghe, L.H., 2017. A synthetic AAV vector enables safe and efficient gene transfer to the mammalian inner ear. Nat. Biotechnol. 35 (3), 280–284. doi:10.1038/nbt.3781.
Lee, H.J., Woo, Y., Hahn, T.W., Jung, Y.M., Jung, Y.J., 2020. Formation and maturation of the phagosome: a key mechanism in innate immunity against intracellular bacterial infection. Microorganisms 8 (9). doi:10.3390/microorganisms8091298.
Lee, J., Nist-Lund, C., Solanes, P., Goldberg, H., Wu, J., Pan, B., Schneider, B.L., Holt, J.R., 2020. Efficient viral transduction in mouse inner ear hair cells with utricle injection and AAV9-PHP.B. Hear. Res. 394, 107882. doi:10.1016/j.heares.2020.107882.
Lester, S.N., Li, K., 2014. Toll-like receptors in antiviral innate immunity. J. Mol. Biol. 426 (6), 1246–1264. doi:10.1016/j.jmb.2013.11.024.
Li, C., Samulski, R.J., 2020. Engineering adeno-associated virus vectors for gene therapy. Nat. Rev. Genet. 21 (4), 255–272. doi:10.1038/s41576-019-0205-4.
Linden, R.M., Ward, P., Giraud, C., Winocour, E., Berns, K.I., 1996a. Site-specific integration by adeno-associated virus. Proc. Natl. Acad. Sci. U. S. A. 93 (21), 11288–11294. doi:10.1073/pnas.93.21.11288.
Linden, R.M., Winocour, E., Berns, K.I., 1996b. The recombination signals for adeno-associated virus site-specific integration. Proc. Natl. Acad. Sci. U. S. A. 93 (15), 7966–7972. doi:10.1073/pnas.93.15.7966.
Liu, Y., Okada, T., Sheykholeslami, K., Shimazaki, K., Nomoto, T., Muramatsu, S., Kanazawa, T., Takeuchi, K., Ajalli, R., Mizukami, H., Kume, A., Ichimura, K., Ozawa, K., 2005. Specific and efficient transduction of Cochlear inner hair cells with recombinant adeno-associated virus type 3 vector. Mol. Ther. J. Am. Soc. Gene Ther. 12 (4), 725–733. doi:10.1016/j.ymthe.2005.03.021.
Majowicz, A., Salas, D., Zabaleta, N., Rodriguez-Garcia, E., Gonzalez-Aseguinolaza, G., Petry, H., Ferreira, V., 2017. Successful repeated hepatic gene delivery in mice and non-human primates achieved by sequential administration of AAV5(ch) and AAV1. Mol. Ther. 25 (8), 1831–1842. doi:10.1016/j.ymthe.2017.05.003.
Manickam, V., Gawande, D.Y., Stothert, A.R., Clayman, A.C., Batalkina, L., Warchol, M.E., Ohlemiller, K.K., Kaur, T., 2023. Macrophages promote repair of inner hair cell ribbon synapses following noise-induced cochlear synaptopathy. J Neurosci. doi:10.1523/JNEUROSCI.1273-22.2023, JN-RM-1273-22. Epub ahead of print. PMID: 36810227.
Manno, C.S., Chew, A.J., Hutchison, S., Larson, P.J., Herzog, R.W., Arruda, V.R., Tai, S.J., Ragni, M.V., Thompson, A., Ozelo, M., Couto, L.B., Leonard, D.G., Johnson, F.A., McClelland, A., Scallan, C., Skarsgard, E., Flake, A.W., Kay, M.A., High, K.A., Glader, B., 2003. AAV-mediated factor IX gene transfer to skeletal muscle in patients with severe hemophilia B. Blood 101 (8), 2963–2972. doi:10.1182/blood-2002-10-3296.
Manno, C.S., Pierce, G.F., Arruda, V.R., Glader, B., Ragni, M., Rasko, J.J., Ozelo, M.C., Hoots, K., Blatt, P., Konkle, B., Dake, M., Kaye, R., Razavi, M., Zajko, A., Zehnder, J., Rustagi, P.K., Nakai, H., Chew, A., Leonard, D., Wright, J.F., Lessard, R.R., Sommer, J.M., Tigges, M., Sabatino, D., Luk, A., Jiang, H., Mingozzi, F., Couto, L., Ertl, H.C., High, K.A., Kay, M.A., 2006. Successful transduction of liver in hemophilia by AAV-Factor IX and limitations imposed by the host immune response. Nat. Med. 12 (3), 342–347. doi:10.1038/nm1358.
Martino, A.T., Markusic, D.M., 2020. Immune response mechanisms against AAV vectors in animal models. Mol. Ther. Methods Clin. Dev. 17, 198–208. doi:10.1016/j.omtm.2019.12.008.
Matern, M., Vijayakumar, S., Margulies, Z., Milon, B., Song, Y., Elkon, R., Zhang, X., Jones, S.M., Hertzano, R., 2017. Gfi1(Cre) mice have early onset progressive hearing loss and induce recombination in numerous inner ear non-hair cells. Sci. Rep. 7, 42079. doi:10.1038/srep42079.
Matsunaga, M., Kita, T., Yamamoto, R., Yamamoto, N., Okano, T., Omori, K., Sakamoto, S., Nakagawa, T., 2020. Initiation of supporting cell activation for hair cell regeneration in the avian auditory epithelium: an explant culture model. Front. Cell. Neurosci. 14, 583994. doi:10.3389/fncel.2020.583994.
Mendell, J.R., Al-Zaidy, S., Shell, R., Arnold, W.D., Rodino-Klapac, L.R., Prior, T.W., Lowes, L., Alfano, L., Berry, K., Church, K., Kissel, J.T., Nagendran, S., L'Italien, J., Sproule, D.M., Wells, C., Cardenas, J.A., Heitzer, M.D., Kaspar, A., Corcoran, S., Braun, L., Likhite, S., Miranda, C., Meyer, K., Foust, K.D., Burghes, A.H.M., Kaspar, B.K., 2017. Single-dose gene-replacement therapy for spinal muscular atrophy. N. Engl. J. Med. 377 (18), 1713–1722. doi:10.1056/NEJMoa1706198.
Mendell, J.R., Campbell, K., Rodino-Klapac, L., Sahenk, Z., Shilling, C., Lewis, S., Bowles, D., Gray, S., Li, C., Galloway, G., Malik, V., Coley, B., Clark, K.R., Li, J., Xiao, X., Samulski, J., McPhee, S.W., Samulski, R.J., Walker, C.M., 2010. Dystrophin immunity in Duchenne's muscular dystrophy. N. Engl. J. Med. 363 (15), 1429–1437. doi:10.1056/NEJMoa1000228.
Meneses, P., Berns, K.I., Winocour, E., 2000. DNA sequence motifs which direct adeno-associated virus site-specific integration in a model system. J. Virol. 74 (13), 6213–6216. doi:10.1128/jvi.74.13.6213-6216.2000.
Milon, B., Shulman, E.D., So, K.S., Cederroth, C.R., Lipford, E.L., Sperber, M., Sellon, J.B., Sarlus, H., Pregernig, G., Shuster, B., Song, Y., Mitra, S., Orvis, J., Margulies, Z., Ogawa, Y., Shults, C., Depireux, D.A., Palermo, A.T., Canlon, B., Burns, J., Elkon, R., Hertzano, R., 2021. A cell-type-specific atlas of the inner ear transcriptional response to acoustic trauma. Cell. Rep. 36 (13), 109758. doi:10.1016/j.celrep.2021.109758.
Mingozzi, F., Anguela, X.M., Pavani, G., Chen, Y., Davidson, R.J., Hui, D.J., Yazicioglu, M., Elkouby, L., Hinderer, C.J., Faella, A., Howard, C., Tai, A., Podsakoff, G.M., Zhou, S., Basner-Tschakarjan, E., Wright, J.F., High, K.A., 2013a. Overcoming preexisting humoral immunity to AAV using capsid decoys. Sci. Transl. Med. 5 (194), 194ra192. doi:10.1126/scitranslmed.3005795.
Mingozzi, F., Buning, H., 2015. Adeno-associated viral vectors at the frontier between tolerance and immunity. Front. Immunol. 6, 120. doi:10.3389/fimmu.2015.00120.
Mingozzi, F., Chen, Y., Edmonson, S.C., Zhou, S., Thurlings, R.M., Tak, P.P., High, K.A., Vervoordeldonk, M.J., 2013b. Prevalence and pharmacological modulation of humoral immunity to AAV vectors in gene transfer to synovial tissue. Gene. Ther. 20 (4), 417–424. doi:10.1038/gt.2012.55.

Mingozzi, F., Chen, Y., Murphy, S.L., Edmonson, S.C., Tai, A., Price, S.D., Metzger, M.E., Zhou, S., Wright, J.F., Donahue, R.E., Dunbar, C.E., High, K.A., 2012. Pharmacological modulation of humoral immunity in a nonhuman primate model of AAV gene transfer for hemophilia B. Mol. Ther. 20 (7), 1410–1416. doi:10.1038/mt.2012.84.

Mingozzi, F., High, K.A., 2013. Immune responses to AAV vectors: overcoming barriers to successful gene therapy. Blood 122 (1), 23–36. doi:10.1182/blood-2013-01-306647.

Moller, M.N., Kirkeby, S., Vikesa, J., Nielsen, F.C., Caye-Thomasen, P., 2015. Gene expression demonstrates an immunological capacity of the human endolymphatic sac. Laryngoscope 125 (8), E269–E275. doi:10.1002/lary.25242.

Monani, U.R., Lorson, C.L., Parsons, D.W., Prior, T.W., Androphy, E.J., Burghes, A.H., McPherson, J.D., 1999. A single nucleotide difference that alters splicing patterns distinguishes the SMA gene SMN1 from the copy gene SMN2. Hum. Mol. Genet. 8 (7), 1177–1183. doi:10.1093/hmg/8.7.1177.

Naso, M.F., Tomkowicz, B., Perry 3rd, W.L., Strohl, W.R., 2017. Adeno-Associated Virus (AAV) as a vector for gene therapy. BioDrugs 31 (4), 317–334. doi:10.1007/s40259-017-0234-5.

Nathwani, A.C., Gray, J.T., Ng, C.Y., Zhou, J., Spence, Y., Waddington, S.N., Tuddenham, E.G., Kemball-Cook, G., McIntosh, J., Boon-Spijker, M., Mertens, K., Davidoff, A.M., 2006. Self-complementary adeno-associated virus vectors containing a novel liver-specific human factor IX expression cassette enable highly efficient transduction of murine and nonhuman primate liver. Blood 107 (7), 2653–2661. doi:10.1182/blood-2005-10-4035.

Nin, F., Hibino, H., Doi, K., Suzuki, T., Hisa, Y., Kurachi, Y., 2008. The endocochlear potential depends on two K^+ diffusion potentials and an electrical barrier in the stria vascularis of the inner ear. Proc. Natl. Acad. Sci. U. S. A. 105 (5), 1751–1756. doi:10.1073/pnas.0711463105.

Okano, T., 2014. Immune system of the inner ear as a novel therapeutic target for sensorineural hearing loss. Front. Pharmacol. 5, 205. doi:10.3389/fphar.2014.00205.

Okano, T., Nakagawa, T., Ito, J., 2010. Distribution of bone marrow-derived cells in the vestibular end organs and the endolymphatic sac. Acta Otolaryngol. (563) 88–94. doi:10.3109/00016489.2010.486803, Suppl.

Okano, T., Nakagawa, T., Kita, T., Kada, S., Yoshimoto, M., Nakahata, T., Ito, J., 2008. Bone marrow-derived cells expressing Iba1 are constitutively present as resident tissue macrophages in the mouse cochlea. J. Neurosci. Res. 86 (8), 1758–1767. doi:10.1002/jnr.21625.

Parham, P., 2021. The Immune System, Fifth edition. Norton & Company, New York, NY W.W..

Penaud-Budloo, M., Francois, A., Clement, N., Ayuso, E., 2018. Pharmacology of recombinant adeno-associated virus production. Mol. Ther. Methods Clin. Dev. 8, 166–180. doi:10.1016/j.omtm.2018.01.002.

Philippidis, A., 2022. Novartis confirms deaths of two patients treated with gene therapy zolgensma. Hum. Gene Ther. 33 (17-18), 842–844. doi:10.1089/hum.2022.29216.bfs.

Poeck, H., Bscheider, M., Gross, O., Finger, K., Roth, S., Rebsamen, M., Hannesschlager, N., Schlee, M., Rothenfusser, S., Barchet, W., Kato, H., Akira, S., Inoue, S., Endres, S., Peschel, C., Hartmann, G., Hornung, V., Ruland, J., 2010. Recognition of RNA virus by RIG-I results in activation of CARD9 and inflammasome signaling for interleukin 1 beta production. Nat. Immunol. 11 (1), 63–69. doi:10.1038/ni.1824.

Rai, V., Wood, M.B., Feng, H., Schabla, N.M., Tu, S., Zuo, J., 2020. The immune response after noise damage in the cochlea is characterized by a heterogeneous mix of adaptive and innate immune cells. Sci. Rep. 10 (1), 15167. doi:10.1038/s41598-020-72181-6.

Rask-Andersen, H., Stahle, J., 1979. Lymphocyte-macrophage activity in the endolymphatic sac. An ultrastructural study of the rugose endolymphatic sac in the guinea pig. ORL J. Otorhinolaryngol. Relat. Spec. 41 (4), 177–192. doi:10.1159/000275458.

Rask-Andersen, H., Stahle, J., 1980. Immunodefence of the inner ear? Lymphocyte-macrophage interaction in the endolymphatic sac. Acta Otolaryngol. 89 (3-4), 283–294. doi:10.3109/00016488009127140.

Roche, P.A., Furuta, K., 2015. The ins and outs of MHC class II-mediated antigen processing and presentation. Nat. Rev. Immunol. 15 (4), 203–216. doi:10.1038/nri3818.

Rogers, G.L., Suzuki, M., Zolotukhin, I., Markusic, D.M., Morel, L.M., Lee, B., Ertl, H.C., Herzog, R.W., 2015. Unique roles of TLR9- and MyD88-dependent and -independent pathways in adaptive immune responses to AAV-mediated gene transfer. J. Innate Immun. 7 (3), 302–314. doi:10.1159/000369273.

Rosendahl Huber, S., van Beek, J., de Jonge, J., Luytjes, W., van Baarle, D., 2014. T cell responses to viral infections - opportunities for Peptide vaccination. Front. Immunol. 5, 171. doi:10.3389/fimmu.2014.00171.

Sabatino, D.E., Mingozzi, F., Hui, D.J., Chen, H., Colosi, P., Ertl, H.C., High, K.A., 2005. Identification of mouse AAV capsid-specific CD8+ T cell epitopes. Mol. Ther. 12 (6), 1023–1033. doi:10.1016/j.ymthe.2005.09.009.

Samulski, R.J., 1993. Adeno-associated virus: integration at a specific chromosomal locus. Curr. Opin. Genet. Dev. 3 (1), 74–80. doi:10.1016/s0959-437x(05)80344-2.

Schartz, N.D., Tenner, A.J., 2020. The good, the bad, and the opportunities of the complement system in neurodegenerative disease. J. Neuroinflamm. 17 (1), 354. doi:10.1186/s12974-020-02024-8.

Schreiber, G., 2020. The role of type I interferons in the pathogenesis and treatment of COVID-19. Front. Immunol. 11, 595739. doi:10.3389/fimmu.2020.595739.

Shishido, S.N., Varahan, S., Yuan, K., Li, X., Fleming, S.D., 2012. Humoral innate immune response and disease. Clin. Immunol. 144 (2), 142–158. doi:10.1016/j.clim.2012.06.002.

Simmonds, P., Aiewsakun, P., 2018. Virus classification - where do you draw the line? Arch. Virol. 163 (8), 2037–2046. doi:10.1007/s00705-018-3938-z.

Smith, C.A., Lowry, O.H., Wu, M.L., 1954. The electrolytes of the labyrinthine fluids. The Laryngoscope 64 (3), 141–153. doi:10.1288/00005537-195403000-00001.

Steines, B., Dickey, D.D., Bergen, J., Excoffon, K.J., Weinstein, J.R., Li, X., Yan, Z., Abou Alaiwa, M.H., Shah, V.S., Bouzek, D.C., Powers, L.S., Gansemer, N.D., Ostedgaard, L.S., Engelhardt, J.F., Stoltz, D.A., Welsh, M.J., Sinn, P.L., Schaffer, D.V., Zabner, J., 2016. CFTR gene transfer with AAV improves early cystic fibrosis pig phenotypes. JCI Insight 1 (14), e88728. doi:10.1172/jci.insight.88728.

Sudres, M., Cire, S., Vasseur, V., Brault, L., Da Rocha, S., Boisgerault, F., Le Bec, C., Gross, D.A., Blouin, V., Ryffel, B., Galy, A., 2012. MyD88 signaling in B cells regulates the production of Th1-dependent antibodies to AAV. Mol. Ther. 20 (8), 1571–1581. doi:10.1038/mt.2012.101.

Summerford, C., Johnson, J.S., Samulski, R.J., 2016. AAVR: a multi-serotype receptor for AAV. Mol. Ther. J. Am. Soc. Gene Ther. 24 (4), 663–666. doi:10.1038/mt.2016.49.

Sung, C.Y.W., Seleme, M.C., Payne, S., Jonjic, S., Hirose, K., Britt, W., 2019. Virus-induced cochlear inflammation in newborn mice alters auditory function. JCI Insight 4 (17). doi:10.1172/jci.insight.128878.

Suzuki, M., Bertin, T.K., Rogers, G.L., Cela, R.G., Zolotukhin, I., Palmer, D.J., Ng, P., Herzog, R.W., Lee, B., 2013. Differential type I interferon-dependent transgene silencing of helper-dependent adenoviral vs. adeno-associated viral vectors *in vivo*. Mol. Ther. 21 (4), 796–805. doi:10.1038/mt.2012.277.

Tan, F., Chu, C., Qi, J., Li, W., You, D., Li, K., Chen, X., Zhao, W., Cheng, C., Liu, X., Qiao, Y., Su, B., He, S., Zhong, C., Li, H., Chai, R., Zhong, G., 2019. AAV-ie enables safe and efficient gene transfer to inner ear cells. Nat. Commun. 10 (1), 3733. doi:10.1038/s41467-019-11687-8.

Thau, L., Asuka, E., Mahajan, K., 2022. Physiology, Opsonization. StatPearls, Treasure Island (FL).

Uribe-Querol, E., Rosales, C., 2017. Control of phagocytosis by microbial pathogens. Front. Immunol. 8, 1368. doi:10.3389/fimmu.2017.01368.

Vandamme, C., Adjali, O., Mingozzi, F., 2017. Unraveling the complex story of immune responses to AAV vectors trial after trial. Hum. Gene. Ther. 28 (11), 1061–1074. doi:10.1089/hum.2017.150.

Verdoodt, D., Peeleman, N., Van Camp, G., Van Rompaey, V., Ponsaerts, P., 2021. Transduction efficiency and immunogenicity of viral vectors for cochlear gene therapy: a systematic review of preclinical animal studies. Front. Cell Neurosci. 15, 728610. doi:10.3389/fncel.2021.728610.

Vidovic, D., Carlon, M.S., da Cunha, M.F., Dekkers, J.F., Hollenhorst, M.I., Bijvelds, M.J., Ramalho, A.S., Van den Haute, C., Ferrante, M., Baekelandt, V., Janssens, H.M., De Boeck, K., Sermet-Gaudelus, I., de Jonge, H.R., Gijsbers, R., Beekman, J.M., Edelman, A., Debyser, Z., 2016. rAAV-CFTRDeltaR rescues the cystic fibrosis phenotype in human intestinal organoids and cystic fibrosis mice. Am. J. Respir. Crit. Care Med. 193 (3), 288–298. doi:10.1164/rccm.201505-0914OC.

Wakabayashi, K., Fujioka, M., Kanzaki, S., Okano, H.J., Shibata, S., Yamashita, D., Masuda, M., Mihara, M., Ohsugi, Y., Ogawa, K., Okano, H., 2010. Blockade of interleukin-6 signaling suppressed cochlear inflammatory response and improved hearing impairment in noise-damaged mice cochlea. Neurosci. Res. 66 (4), 345–352. doi:10.1016/j.neures.2009.12.008.

Wang, D., Tai, P.W.L., Gao, G., 2019. Adeno-associated virus vector as a platform for gene therapy delivery. Nat. Rev. Drug Discov. 18 (5), 358–378. doi:10.1038/s41573-019-0012-9.

Wang, G.P., Guo, J.Y., Peng, Z., Liu, Y.Y., Xie, J., Gong, S.S., 2014. Adeno-associated virus-mediated gene transfer targeting normal and traumatized mouse utricle. Gene Ther. 21 (11), 958–966. doi:10.1038/gt.2014.73.

Wang, J.Q., Jeelall, Y.S., Ferguson, L.L., Horikawa, K., 2014. Toll-like receptors and cancer: MYD88 mutation and inflammation. Front. Immunol. 5, 367. doi:10.3389/fimmu.2014.00367.

Weitzman, M.D., Kyostio, S.R., Kotin, R.M., Owens, R.A., 1994. Adeno-associated virus (AAV) Rep proteins mediate complex formation between AAV DNA and its integration site in human DNA. Proc. Natl. Acad. Sci. U. S. A. 91 (13), 5808–5812. doi:10.1073/pnas.91.13.5808.

Wilson, J.M., Flotte, T.R., 2020. Moving forward after two deaths in a gene therapy trial of myotubular myopathy. Hum. Gene. Ther. 31 (13-14), 695–696. doi:10.1089/hum.2020.182.

Wu, Z., Asokan, A., Samulski, R.J., 2006. Adeno-associated virus serotypes: vector toolkit for human gene therapy. Mol. Ther. J. Am. Soc. Gene Ther. 14 (3), 316–327. doi:10.1016/j.ymthe.2006.05.009.

Xiao, X., Li, J., Samulski, R.J., 1996. Efficient long-term gene transfer into muscle tissue of immunocompetent mice by adeno-associated virus vector. J. Virol. 70 (11), 8098–8108. doi:10.1128/JVI.70.11.8098-8108.1996.

Yamashita, T., Zheng, F., Finkelstein, D., Kellard, Z., Carter, R., Rosencrance, C.D., Sugino, K., Easton, J., Gawad, C., Zuo, J., 2018. High-resolution transcriptional dissection of *in vivo* Atoh1-mediated hair cell conversion in mature cochleae identifies Isl1 as a co-reprogramming factor. PLOS Genet. 14 (7), e1007552. doi:10.1371/journal.pgen.1007552.

Yan, Z., Zak, R., Zhang, Y., Engelhardt, J.F., 2005. Inverted terminal repeat sequences are important for intermolecular recombination and circularization of adeno-associated virus genomes. J. Virol. 79 (1), 364–379. doi:10.1128/JVI.79.1.364-379.2005.

Yang, W., Vethanayagam, R.R., Dong, Y., Cai, Q., Hu, B.H., 2015. Activation of the antigen presentation function of mononuclear phagocyte populations associated with the basilar membrane of the cochlea after acoustic overstimulation. Neuroscience 303, 1–15. doi:10.1016/j.neuroscience.2015.05.081.

Zdebik, A.A., Wangemann, P., Jentsch, T.J., 2009. Potassium ion movement in the

inner ear: insights from genetic disease and mouse models. Physiology 24, 307–316. doi:10.1152/physiol.00018.2009.

Zhang, W., Dai, M., Fridberger, A., Hassan, A., Degagne, J., Neng, L., Zhang, F., He, W., Ren, T., Trune, D., Auer, M., Shi, X., 2012. Perivascular-resident macrophage-like melanocytes in the inner ear are essential for the integrity of the intrastrial fluid-blood barrier. Proc. Natl. Acad. Sci. U. S. A. 109 (26), 10388–10393. doi:10.1073/pnas.1205210109.

Zincarelli, C., Soltys, S., Rengo, G., Rabinowitz, J.E., 2008. Analysis of AAV serotypes 1-9 mediated gene expression and tropism in mice after systemic injection. Mol. Ther. J. Am. Soc. Gene Ther. 16 (6), 1073–1080. doi:10.1038/mt.2008.76.