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Implications of the innate immune response to adenovirus and adenoviral vectors

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Abstract

Adenovirus (AdV) is a common cause of respiratory illness in both children and adults. Respiratory symptoms can range from those of the common cold to severe pneumonia. Infection can also cause significant disease in the immunocompromised and among immunocompetent subjects in close quarters. Fortunately, infection with AdV in the normal host is generally mild. This is one reason why its initial use as a gene-therapy vector appeared to be so promising. Unfortunately, both innate and adaptive responses to the virus have limited the development of AdV vectors as a tool of gene therapy by increasing toxicity and limiting duration of transgene expression. This article will focus on the innate immune response to infection with wild-type AdV and exposure to AdV gene-therapy vectors. As much of the known information relates to the pulmonary inflammatory response, this organ system will be emphasized. This article will also discuss how that understanding has led to the creation of new vectors for use in gene therapy.

Keywords

adenovirus; cytokines; innate immunity; vector

Up until the advent of gene therapy, interest in adenovirus (AdV) and its related diseases was primarily the concern of neonatologists and those involved in the care of immunosuppressed patients. Thus, the focus was on vaccination and early attempts at therapeutics. This changed with the introduction of the idea that “good DNA could be used to replace defective DNA in those who suffer from genetic defects”, first proposed by Rogers in 1970 [1]. However, it was not until 20 years later that the first successful human implementation of this new science occurred [2]. After this initial success, this promising new field continued to blossom for almost a decade until the unfortunate death of a volunteer in a gene-therapy research trial [3]. The volunteer was an 18-year-old patient with ornithine transcarbamylase deficiency. Although there were other potential confounding

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factors, he was the first research volunteer whose death was attributed to gene therapy. The research volunteer had been administered a large dose (6×10^{11} viral particles/kg) of an early-generation AdV vector, which triggered a massive immune response associated with multiorgan failure and eventual death [4]. This case disrupted the field of gene therapy and led to a governmental investigation of both the scientists involved and the university, ultimately, resulting in stricter oversight and overall reform of the field [5].

Until the volunteer's death, the use of AdV vectors was considered the most promising gene-transfer technology because they were easily produced, were not mutagenic and were capable of transducing a wide variety of cells [6]. The concern that the death of the volunteer may have been owing to activation of the innate immune response by the large dose of AdV vector used has been partially responsible for attempts to create newer generations of vectors, including modification of both the DNA and the capsid of the virus [7,8]. Their attempts have been guided by the evolving knowledge of the immune response to natural infection by AdV.

The host response to AdV infection involves both the innate and the adaptive immune systems [9]. The innate immune response governs the host's immediate response to infection and it has evolved to rapidly detect and eliminate numerous pathogens. The innate immune response is fixed, and detection is limited because the receptors of the innate immune system are fully encoded in the genome and do not undergo somatic recombination. However, the adaptive immune response is not under this constraint. Owing to receptor gene somatic recombination, the response of the major players of the adaptive immune response – the antigen-presenting cell, the T lymphocyte and the B lymphocyte – can be focused to optimally recognize the specific pathogens to which they are exposed. The preservation of the immunologic memory that results from this process assists in prevention of reinfection or serious illness upon re-exposure to the same or similar pathogens [9].

In the case of AdV vectors for use in gene therapy, current evidence suggests that innate immune responses are responsible for the dose-limiting toxicity and that the adaptive immune responses, with elimination of transduced cells, limit the duration of transgene expression. Using information derived from wild-type infection, and from experience using earlier-generation vectors, scientists have endeavored to create a new 'gutless' vector, which has almost all viral DNA removed in order to attempt to minimize the adaptive immune response. Indirectly, this may decrease the initial innate immune response, as a lower dose of vector may be needed for prolonged gene expression. Several excellent reviews regarding the adaptive response to AdV, particularly gene-therapy vectors, are available [10,11]. However, this article will summarize the current understanding of the innate immune response to AdV infection in the respiratory system. Primarily, the focus will be on how the innate immune response is activated by AdV. Finally, this article will address how gutless vectors have been modified in order to avoid provocation of that response. It is noted, and will be discussed, that this modification may only indirectly decrease acute toxicity by reducing the initial dose needed to facilitate long-term expression. Additional adjunctive therapies used to diminish immune responses to AdV will also be discussed.

Adenovirus structure & serotypes

Adenovirus was first cultured and reported as a distinct viral agent in 1953 [12]. AdV infections cause a broad spectrum of illness in immunocompromised and in immunocompetent hosts. AdV is the cause of a large number of acute febrile respiratory syndromes among military recruits [13]. It is also a cause of ocular, respiratory and gastrointestinal infections in the general population. Structurally, AdV is nonenveloped, regular and icosahedral. It is estimated to be 65–90 nm in diameter [14–16]. The virion has

20 triangular faces and 12 vertices, with 240 hexons and 12 pentons. Each vertex contains a penton complex consisting of the penton base and fiber proteins. Inside the capsid is a single molecule of dsDNA with a molecular weight of $2.0\text{--}2.4 \times 10^7$ (or ~35 kb in length). Antigenic determinants that are important in the serologic classification of AdV are inherent to the hexon, penton base and fiber. All human AdV serotypes share a crossreacting group antigen that is carried on the inner surface of the hexon capsomere [17]. To date, 55 serotypes of human AdV have been recognized [18–20] and grouped into seven species (formerly termed ‘subgenera’) on the basis of their hemagglutinating properties and biophysical and biochemical criteria and, more recently, DNA sequence homology: species A, B (subdivided into subspecies B1 and B2), C, D, E, F and G [20,21]. Subgroup identification is of more than simply academic interest, as the type of infections caused by the virus depends on the subgroup to which the virus belongs [22,23]. This is also of relevance to the material that follows, as the innate immune response may also depend on the AdV subgroup studied [24,25].

Innate immune system

There are many components of the innate immune system that act to prevent infection upon exposure to infectious agents, including viruses. These can be generally classified into mechanical defenses, anti-infective chemical defenses, cellular defenses and chemokine/cytokine defenses. A detailed description of these components and their role in AdV infection follows.

Mechanical defenses

Mucociliary clearance and coughing are the two mechanical methods of pathogen clearance in the lung. Mucociliary clearance is a process that involves distal to proximal transport of a bilayer of mucus and a periciliary fluid by cilia, collectively referred to as the airway surface fluid. The composition of the bilayer allows infectious particles to be bound in the surface mucus, while the cilia are able to function in a lower-density layer. This bilayer increases the efficiency of transport and achieves clearance of infectious particles from the peripheral airways in relatively short periods of time [26]. Clearance of bacteria from peripheral airways by mucus transport may require up to 6 h. Bacterial replication is inhibited for 3–6 h by various endogenous antimicrobial factors, including lactoferrin, lysozyme and defensins [27]. Conversely, viruses require cellular machinery to reproduce, and so mucosal inhibitors act by interfering with the ability of these organisms to infect cells.

There are several inhibitors of viral infectivity present in mucus. The structure of mucus contains multiple sialic acid residues. These residues compete with cellular receptors used by viruses, such as influenza virus, and, therefore, decrease virus infectivity. Mucus protease inhibitor prevents the action of airway tryptase clara, which enhances influenza A virus infectivity [28]. The role of these substances in protection from AdV infection has not been established, but it is likely that sialic acid residues in mucus inhibit viral infectivity in a similar manner since sialic acid is involved in the attachment of some subgroup D AdV types to epithelial cells [29–31]. Also, an artificially synthesized sialic acid derivative inhibits the subgroup D AdV 37 to corneal epithelia cells [32]. With regards to subgroup C AdV vectors, the mucus bilayer is perhaps a limiting factor for optimal AdV gene transfer [33]. In addition, there is charge-dependent repulsion between AdV-5 group C capsid protein and cell surface sialic acid, which can be diminished and, thus, infectivity enhanced by neuraminidase [29,34–36]. Therefore, the effect of sialic acid on AdV infection depends on the AdV type and cellular environment, in some cases enhancing and in others inhibiting this process.

Adenovirus has been shown to be inefficient in targeting proximal human and murine airway epithelium [37]. One important reason for this inefficiency appears to be the lack of appropriate AdV receptors on ciliated airway epithelial cells [38]. These interactions will be further discussed later in this article.

Chemical defenses

Antimicrobial peptides—Generally, particles of less than 5 μm bypass the mechanical defenses and gain access to the terminal airways. Therefore, additional defense mechanisms are required to maintain lung sterility. Important classes of antimicrobial compounds are the cationic antimicrobial peptides (CAMPs). These are peptides present intracellularly in cellular phagocytic components of the innate immune system, but they are also secreted into the airway by epithelial cells. They contain three major classes of molecules: the defensins produced by epithelial cells, neutrophils, monocytes and macrophages and dendritic cells [39,40], the cathelicidins produced by neutrophils, mast cells and epithelial cells [41–43], and the thrombocidins, which are produced by platelets [44].

Defensins—Defensins are single-chain, strongly cationic peptides, with molecular weights of 3–4.5 kDa [45]. They are divided into two classes, α - and β -defensins, based on their chemical structure. The α -defensins HNP-1–4 are produced by neutrophils, while the α -defensins HD-5 and -6 are both produced by the intestinal Paneth cells, but HD5 is also found in the female reproductive tract and has been detected in nasal and bronchial epithelial cells [46]. β -defensins HBD-1–3 are produced by epithelial cells of many organ systems, while HBD-4 is primarily expressed in the testis and gastric antrum [47–53]. These molecules have a broad spectrum of microbicidal activity against Gram-positive and Gram-negative bacteria, mycobacteria, fungi and certain enveloped viruses [45,54,55]. The antibacterial (but not antiviral) activity of defensins is dependent upon low salt concentrations [51,53,56,57]. This has implications in patients with cystic fibrosis in whom the salt concentration is altered owing to the genetic defect.

Although AdV is nonenveloped, defensins have activity against this virus. The human α -defensin HNP-1 is present in bronchoalveolar lavage (BAL) fluid and inhibits AdV-5 vector infection *in vitro* by 95% [58]. The α -defensin HD5 as well as the β -defensin HBD-1 also reduce AdV-5 vector infectivity *in vitro* by three- to five-fold [59]. Some of the defensins, especially HBD-2, are upregulated by various cytokines, including IL-1 β [51,60]. Likewise, production of cytokines, such as IL-6 and IL-8, can be induced by defensins [61]. This provides for amplification of the antiviral response during infection. Defensins inhibited infectivity of wild-type AdV. The mechanism of this inhibition has been shown to be due to α -defensin HD5 and HNP1 blockade of AdV uncoating events, including the release of the endosomal protein VI, thus preventing genome exposure and nuclear entry [57,62,63].

Cathelicidins—Cathelicidins are antimicrobial peptides found exclusively in mammals. *In vitro* and *in vivo* studies indicate that they are effector molecules of mammalian innate immunity. Although many different cathelicidins have been identified in mammals, only one has been identified in humans. The first human cathelicidin was cloned from cDNA isolated from human bone marrow as FALL-39 [64]. It is identical to the independently identified antimicrobial domain of hCAP-18 produced by neutrophils [65]. This peptide is cleaved to form a C-terminal fragment with antimicrobial activity, known as LL-37 [66]. The C-terminus of this peptide fragment binds and inactivates lipopolysaccharide [67]. LL-37/hCAP18 is present in neutrophil granules [65], and is produced by bone marrow and testis [64], epithelial cells of the skin [68], and respiratory [43] and squamous epithelia of human mouth, tongue, esophagus, cervix and vagina [69].

Cathelicidins have activity against bacteria, fungi and viruses, which varies by the species studied [43,70]. Data support a role for cathelicidins in the inhibition of orthopox virus (vaccinia) replication both *in vitro* and *in vivo* [71]. The lack of plasmacytoid dendritic cell recruitment, together with the missing upregulation of cathelicidin LL37 in atopic dermatitis lesions, is considered relevant for an atopic dermatitis patient's susceptibility to eczema vaccinatum [72]. LL-37 has some activity against herpes simplex virus (HSV)-1 and -2, *in vitro*, but there are no reports for testing against AdV [73]. These peptides may have an indirect activity against AdV as they are upregulated by IL-6 [69] and IFN- γ [74], and cathelicidins are chemotactic for neutrophils, monocytes and T cells [74,75]. However, LL-37/hCAP18 was not upregulated in human colon epithelial cells stimulated with cytokines playing a role in intestinal inflammatory responses, including TNF- α , IL-1 α , IFN- γ and IL-6 [76]. Thus, LL-37/hCAP18 appears to be differentially regulated among different cell types. Cathelicidins are probably partially responsible for the inflammatory infiltrate seen during AdV infection, since there is evidence that cathelicidins are upregulated by cytokines induced by AdV, and because cathelicidins are chemotactic.

Thrombicidins—Thrombicidins are antimicrobial peptides released from platelets stimulated with thrombin or found in platelet extracts. They are also released when platelets are exposed to infectious agents. Several of these peptides are either structurally similar to, or identical to, known chemokines [77]. Studies of peptides have demonstrated *in vitro*, and in some cases *in vivo*, activity against a few bacterial species and fungal species [44,77–79]. However, there are as yet no studies of the direct antiviral activities of thrombicidins.

Pulmonary surfactants—Although pulmonary surfactants' primary role is to facilitate lung expansion by reducing surface tension, they also play a role in lung defense against infections. Two surfactant proteins, SP-A and SP-D, have antimicrobial activity. In this regard, they are classified as collectins or collagenous C-type lectins. These proteins consist of oligomers of trimeric subunits the subunits contain C-type lectin carbohydrate-recognition domains [80]. These domains bind to a number of different organisms, including bacteria and fungi, and decrease virulence both by inhibiting infectivity and by enhancing phagocytosis by cellular components of the innate immune system [80–84].

The lung collectins show specific interactions with various respiratory viruses. Purified SP-A and SP-D inhibit infectivity and hemagglutination activity of influenza A virus *in vitro* [85,86]. Collectins also inhibit the infectivity of respiratory syncytial virus (RSV) [87] and rotavirus [88]. SP-A also promotes the phagocytosis of HSV by rat alveolar macrophages [89].

Animal studies using SP-A- or SP-B-deficient mice have verified an *in vivo* effect on clearance of several of these microbes. Clearance of bacterial pathogens, as well as viral pathogens, such as RSV and influenza A virus, was deficient in SP-A-deficient mice [90]. SP-D enhanced the phagocytosis and pulmonary clearance of RSV in mice [91]. SP-D-deficient mice showed decreased viral clearance of certain strains of influenza A virus, and increased production of inflammatory cytokines in response to viral challenge. Viral clearance of strains of influenza A virus and the cytokine response were both normalized by the coadministration of recombinant SP-D [92]. Of particular interest to this article, clearance of AdV DNA from the lung and uptake of fluorescent-labeled AdV vector by alveolar macrophages was decreased in SP-A-deficient mice. Supplementation with SP-A enhanced viral clearance and inhibited lung inflammation during pulmonary adenoviral infection, confirming the importance of SP-A in antiviral host defense [93].

Cellular defenses

Macrophages—Cellular defenses of the innate immune system include both resident cells and those recruited from the bloodstream. Alveolar macrophages are the most prominent resident cells that engulf and kill infectious agents. They also provide a link to the adaptive immune system and function as antigen-presenting cells, although less effectively than other cells [94–96]. Macrophages have a pivotal role in immune surveillance of the respiratory tract, initiation of anti-infective inflammation and regulation of potentially harmful inflammatory responses. Alveolar macrophages make up 85% of cells recovered in BAL [97]. Alveolar macrophages play an important role in the elimination of AdV vectors from the lung. Alveolar macrophages recovered from the mouse lung 30 min after intratracheal administration of an AdV vector showed large amounts of vector genome, whereas much less was evident in alveolar macrophages recovered after 24 h [98]. In a murine model, AdV labeled with a fluorescent dye was very rapidly (~1 min) localized within the alveolar macrophages [99]. Although best studied in epithelial cells [100], internalization of AdV by mononuclear phagocytes is believed to occur by integrin receptor-mediated endocytosis [101,102].

Alveolar macrophages were also identified as a cell source of initial cytokine signaling by AdV. At 30 min after infection, alveolar macrophages expressed mRNA for TNF- α and IL-6, but airway epithelial or vascular endothelial cells did not. Blockage of virus uptake prior to internalization in the alveolar macrophages completely blocked TNF- α expression [99]. Other products released by macrophages include IL-12 (activates NK cells), LTB₄, toxic oxygen species, IL-1 (T-cell stimulatory and proinflammatory cytokine) and antibacterial products, such as lactoferrin, lysozyme and defensins [99,103]. TNF, IL-1 and IL-6 are considered primary activators of the immune system. Although this activation is mostly caused by response to detection of the capsid proteins, AdV and vector DNA alone activate the immune response via Toll-like receptors (TLRs) [104]. Mice with impaired TLR9 receptors have significantly reduced IL-6 secretion compared with wild-type mice when exposed to HD AdV vectors [105].

Dendritic cells—Cells of similar lineage, the dendritic cells, also function in the respiratory tract, and are more like tissue macrophages present in other organ systems. They are less efficient at phagocytosis, but more efficient at antigen presentation than alveolar macrophages. They are indispensable to the initiation of the adaptive immune response. Tobacco smoke decreases the number of dendritic cells in lung tissue, and chronic tobacco exposure impairs the immune response against AdV [106]. This may be relevant as smokers appear to be at risk for pneumonia due to wild-type AdV [107]. Antigen presentation by murine dendritic cells is NF- κ B dependent, and NF- κ B inhibition blocks dendritic cell AdV antigen presentation and has a marked immunosuppressive effect *in vivo* [108]. Preferential activation of dendritic cells and macrophages may account for AdV inflammatory responses *in vivo*. Elimination of tissue macrophages and splenic dendritic cells *in vivo* considerably reduced the early release of IL-6, IL-12 and TNF- α , and significantly blocked the specific cellular immune response to AdV [109]. It is known that viral gene expression is not necessary for dendritic cell activation [110,111]. Viral capsid proteins, the penton, hexon and fiber, have all been shown to trigger activation of dendritic cells [112–114]. However, dendritic cells are also activated through TLRs during exposure to AdV infection [104]. When peripheral blood monocyte cells with or without plasmacytoid dendritic cells and purified plasmacytoid dendritic cells were exposed to AdV, only the purified plasmacytoid dendritic cells produced large levels of IFN- α , and this production was blocked when the cells were treated with chemicals to block TLR activation [115]. It also appears that the production of interferon (IFN) by dendritic cells leads to their own maturation [116]. Which mechanism is most responsible for dendritic cell activation by AdV is currently under study,

but evidence is accumulating that TLR-independent activation of dendritic cells by AdV is important [117,118].

Neutrophils—Neutrophils are also important in the response to infectious agents, and are probably important to AdV. They internalize AdV in the presence of complement or IgG, and are attracted to the lung following infection with AdV [119]. This is partially due to release of various cytokines, including IL-8, by macrophages. IL-8 is a major neutrophil chemotactic factor in the lung [120]. Intravenously administered AdV significantly increased leukocyte rolling and adhesion in the liver within minutes of transduction. P-selectin, α_4 -integrin and E-selectin are important in this process, as blockade of these receptors inhibited leukocyte rolling and subsequent adhesion. Depletion of circulating neutrophils eliminated leukocyte rolling and adhesion [121]. MIP-2 antibody and neutrophil depletion diminished hepatic injury in human AdV vector-exposed mice, as determined by both reduced serum aspartate aminotransferase and alanine aminotransferase levels and histology [122]. This suggests that early tissue injury is largely owing to chemokine production and neutrophil recruitment. One strategy to prevent AdV-mediated inflammation and, therefore, tissue injury would be to interfere with chemokine or neutrophil function.

Epithelial cells—The airway epithelial cells not only function as a passive barrier to infectious particles but also actively participate in the innate immune response to foreign antigens. This is particularly important in AdV infections because the epithelium is the major site for AdV replication. The immune response of epithelia to infection and antigen exposure consists of an increase in the release of antimicrobial peptides into the lumen of the airways, and the release of chemokines and cytokines into the submucosa, which initiate an inflammatory reaction. This results in the recruitment of phagocytes that help clear the invaders, and of dendritic cells and lymphocytes, which facilitate an adaptive immune response. Activation of TLRs on epithelial cells has now been shown to be involved in the regulation of expression of a variety of genes, including those encoding cytokines, chemokines and antimicrobial peptides [123]. TLR-mediated responses to AdV have been demonstrated in murine corneal epithelium [124]. However, AdV infection of the cornea resulting in keratitis was similar between wild-type mice and mice without TLR9. Also, the virally induced expression of chemokines and leukocyte infiltration could be blocked with RGD peptides. These results suggest that viral capsid–host cell integrin interactions are more important than TLR-mediated responses in the murine corneal epithelium [125].

There are other mechanisms whereby components of the innate immune system, in this case the lung epithelia, respond to AdV infection. AdV vector infection of A549 respiratory epithelial cells induces a significant expression of ICAM-1 and increased CD18-dependent adhesion of activated neutrophils [126].

This may be partially responsible for the initial inflammation seen in animal models of AdV infection, although direct stimulation of cytokines both from lung epithelia and alveolar macrophages also plays a role. Intratracheal administration of AdV genotypes 3p and 7h in mice caused a robust inflammatory response in the lung, which initially consisted of neutrophilic and monocytic alveolar infiltration. There was also mild peribronchial and perivascular inflammation. At early time periods after exposure, neutrophils were the predominant cell type recovered by alveolar lavage. This correlated with increased levels of neutrophilic chemokines, such as MIP-2 and KC (mouse IL-8 homologs) in lung tissue. Elevation of other pro-inflammatory cytokines, such as IL-1 β , TNF- β , IFN- γ and IL-12, were also noted [127].

It is likely that direct infection or stimulation of distal epithelia is responsible for many of these changes, as the peribronchial inflammation was minimal and because proximal

bronchial human airway epithelial cells are resistant to infection by AdV, at least as determined for AdV-5 vectors [38]. Infection of epithelia by AdV involves two steps. First, the globular head of the AdV fiber attaches to either the coxsackie and AdV receptor, the MHC class I α -2 domain or the CD46 receptor depending on the AdV subtype [128–131]. Then the penton base of AdV binds to the $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrins or $\alpha_v\beta_1$ integrins, which are important for virus internalization [102,132,133]. The resistance of the bronchial epithelia to AdV infection appears to be due to a lack of the appropriate receptors on the apical membrane. Coxsackie and AdV receptor and MHC I are polarized to the less accessible basolateral membrane [134], and $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrins are also minimally expressed on the apical plasma membrane of proximal airway epithelial cells [135]. Several comprehensive reviews of AdV receptors are available [136,137].

It is important to note that these restrictions are not likely to be as important in the distal human lung, specifically, in the alveolar epithelium. Human alveolar epithelial cell lines are easily infected by both type 5 and type 7 wild-type AdV [24]. Also, human lung tissue slices support wild-type AdV 7 virus replication, infection of the alveolar epithelia and an innate immune response, as evidenced by increased IL-8 release [138].

Cytokines & AdV

Adenovirus induces different clinical manifestations, ranging from unapparent infection or benign upper respiratory tract disease to necrotizing bronchiolitis or even disseminated fatal disease in immunocompromised patients. AdVs are cytopathic in permissive cell cultures and the inflammation in affected areas frequently extends beyond the necrotic epithelia, where AdV inclusions are most prominent. This suggests that additional mechanisms might be involved in the pathogenesis of tissue damage [139–141]. Activation of the immune system and the generation of numerous chemokines and cytokines clearly play a role in activation of inflammation and, therefore, could play a major role in the pathogenesis of tissue damage. Although there is a distinction between chemotactic cytokines (chemokines) and nonchemotactic cytokines by their ability to induce cell migration, their functions are interrelated. For example, chemokines not only attract inflammatory cells, they also activate them, and may cause them to proliferate. Also, while nonchemotactic cytokines may also have activating and proliferative effects, they also indirectly stimulate chemotaxis by stimulating the release of chemotactic cytokines. Both chemotactic and nonchemotactic cytokines have been shown to play an important role in pulmonary host defense. These include TNF- α , IL-1, IL-6, IL-8, IL-10, IL-12, IFN- γ and granulocyte colony-stimulating factor [142]. There is evidence that the cytokine response by itself is important in the consequences of infection with AdV. Increased concentrations of IL-6, IL-8 and TNF- α were associated with hypoperfusion, febrile peaks, tonic-clonic seizures and septic shock, and were significantly associated with the severity of AdV infection in children [143]. The activation of the innate immune response also occurs in the absence of virus gene transcription [24,144,145]. The alveolar macrophages are proposed to be the source of initial cytokine signaling after acute AdV respiratory tract infections. In a murine model, 30 min after infection, alveolar macrophages but not airway epithelial or vascular endothelial cells expressed mRNA for TNF- α and IL-6 [99]. The signal for initiation of TNF- α expression during AdV exposure to RAW264.7, a macrophage cell line, requires virion internalization and probably occurs during or subsequent to endosome acidification, as blockage of these specific steps of passage of the virus completely blocked TNF- α expression [99]. There is some uncertainty as to whether endosome acidification is required, as the agent to prevent this process, chloroquine, inhibits signaling pathways known to be important for cytokine production [146]. Other cells of the immune system also clearly participate in the initial innate immune response, as chemokines, particularly IL-8, are induced in airway epithelial cells by AdV in culture and in human tissue [24,25,138]. The innate immune response of

alveolar epithelial cells does not appear to require triggering by macrophages, as IL-8 is induced in pure epithelial cell cultures in the absence of macrophages and IL-8 is induced in a human slice model where macrophages are not prominent in the preparation [24,25,138].

IL-1

IL-1 plays a role in the early inflammatory response seen after AdV infection. In a mouse model, intranasal inoculation of wild-type AdV-5 produced pneumonia, even though the virus did not replicate. Assays showed the appearance of IL-1, TNF- α and IL-6 in mouse lungs concomitant with the development of an early-phase infiltration with lymphocytes and monocytes/macrophages [147]. IL-1 is also the major mediator of a very early inflammatory response to AdV vector in the brain [148]. IL-1 β may also play a role in progressive fibrosis and tissue remodeling in mice through the induction of profibrotic cytokines PGDF and TGF- β 1 [149]. A key event in virus-induced inflammation is the local activation of endothelial cells, as indicated by the expression of adhesion molecules, such as ICAM-1, VCAM-1 and E-selectin. Fluids from AdV type 37-infected respiratory and ocular epithelial cells activated ICAM-1 and, to a lesser extent, VCAM-1 expression in cultured human umbilical vein cells [150]. Blocking studies with anticytokine antibodies implicated IL-1 α as the epithelial cell-derived factor that activated ICAM-1 expression [150]. AdV-infected A549 respiratory cells treated with an IL-1 receptor antagonist (IRAP) expressed 75% less IL-8 than untreated cells, whereas IRAP pretreatment of TNF- α -stimulated cells did not affect IL-8 production [151]. Thus, IL-8 production by AdV vector-infected cells occurs partly through IL-1 activation that can be downregulated by IRAP. It is not known whether IL-1 is important in IL-8 induction by wild-type AdV. Finally, AdV vectors have been shown to induce IL-1 in macrophages through interaction with β_3 integrins. Induction of IL-1 does not involve traditional known signaling pathways [152].

IL-6

IL-6 is another cytokine that has been associated with the immune response following AdV infection. IL-6 is produced by vascular endothelial cells, mononuclear phagocytes, fibroblasts and activated T lymphocytes in response to a variety of stimuli and is referred to as the global response marker [153]. Nijsten *et al.* reported that IL-6 levels increase earlier in the acute-phase response than those of other proteins, such as C-reactive protein and α -1 antitrypsin, and are related to the generation of fever [154]. IL-6 may act as a hepatocyte-stimulating factor and induces various acute-phase proteins in the liver cells [155]. When classified according to the clinical findings and outcome, IL-6 was detected in the groups with severe and fatal AdV infections in children, and not in the group classified as moderate [143]. Increased concentrations of IL-6 were associated with hypoperfusion, febrile peaks, tonic-clonic seizures and septic shock in these children. High serum values are associated with the severity of AdV infection. In a study comparing 106 children with AdV, influenza or RSV infections, the IL-6 concentration was greater than 50 pg/ml in 78.6% of patients with AdV infections, compared with 15.8% in patients with influenza and none in patients with RSV infections. IL-6 was again associated with the severity of AdV infection and correlated with serum concentrations of C-reactive protein in the group with AdV infection [156]. Presumably, IL-6 plays a dual effect during viral infections – it may stimulate immune defenses against infected cells and may participate in tissue damage [157].

IL-8

The importance of IL-8 in acute AdV infection is suggested by the consistent finding of prominent neutrophilic peribronchial and alveolar infiltration early in the disease process in a number of animal models [158–161]. Inflammation also occurred under conditions in which the virus did not replicate [147]. This is important, as IL-8 is the major neutrophil chemotactic factor in the lung [120]. There are data suggesting that IL-8 has a role in the

pathogenesis of naturally acquired acute AdV infection in humans. In children with AdV pneumonia, serum IL-8 levels correlate with clinical outcome [143]. The highest levels are seen in patients with a fatal outcome and approach values seen in patients with sepsis. Both TNF- α and IL-1, produced by alveolar macrophages, are potent inducers of IL-8 production by several cell types, including alveolar macrophages, type II epithelial cells and lung fibroblasts [120]. AdV strains and vectors vary in their ability to induce IL-8 and cause lung disease. Wild-type AdV-5 does not induce IL-8 release *in vitro* or cause pneumonia in immunocompetent humans. Both E1A-deficient AdV-5 vectors and wild-type AdV-7 induce IL-8 release and pneumonitis [13,24,162–166]. It is likely that AdV gene expression is not necessary for IL-8 induction, as induction occurs prior to AdV protein expression in AdV-7-infected cells, and AdV protein expression is minimal in gene-therapy vectors. Therefore, it is likely that induction occurs through the interaction of surface proteins with cellular receptors, which are known for AdV-5 but not entirely known for AdV-7. This could result in signaling pathway induction resulting in *IL-8* gene expression. We, and others, have demonstrated p42/44 MAPK activation with exposure of cells to both virus types. We have confirmed that activation of this kinase is essential for IL-8 induction by AdV-7 [25]. The role of other signaling pathways in IL-8 induction by AdV is not known. Also unexamined is the mechanism whereby modification of wild-type AdV-5 for gene therapy results in a vector that is capable of inducing IL-8. A similarly modified AdV-7 replication-deficient vector has been constructed, but has not been tested for IL-8 or signal pathway induction.

Work by Hogg and coworkers suggests that latent AdV may act as a coactivator of the *IL-8* gene, predisposing to chronic inflammation and the development of chronic obstructive pulmonary disease (COPD) in some patients. In their studies, increased amounts of AdV–DNA, specifically the AdV–E1A proteins, were detected in the lungs of patients with COPD compared with age-matched asymptomatic patients with similar smoking exposures [167,168]. Additional studies showed that quantitative regional expression of the AdV–E1A proteins in lung epithelial tissue also correlated with the number of inflammatory cells and the amount of lung destruction as determined by computed tomography [169]. *In vitro* studies, using lung epithelial cell lines, demonstrated that stable transfection with AdV–E1A proteins enhanced lipopolysaccharide-induced expression of IL-8 through NF- κ B [170,171]. Thus, the presumed mechanism of AdV enhancement of COPD is amplification of the cytokine response, specifically IL-8 to external stimuli, such as lipopolysaccharide or cigarette smoke. There remains uncertainty about the role of AdV in COPD pathogenesis, as a recent study has shown a low incidence of E1A RNA expression and E1A DNA in patients with COPD [172].

IL-12

IL-12 is a heterodimeric protein consisting of two subunits (p35 and p40) and plays an important role in promoting Th1-type immune responses. It serves as the major signal for IFN- γ expression from T cells and NK cells [173]. Therefore it plays a role in linking the innate and adaptive immune systems.

IL-12 is induced during both wild-type AdV infection and AdV vector exposure. In a mouse model, after infection with mouse wild-type AdV, message for the p40 component of IL-12 was transiently increased shortly after infection [174]. IL-12 was expressed mainly by macrophages. AdV vector also stimulates IL-12. AdV vector infection of mouse dendritic cells stimulated IL-12 release, or IL-12-dependent dendritic cell activation of NK and T cells [175–177]. As well as this, an increased IL-12 response to AdV vector infection in BALB/c mice correlated with increased AdV vector clearance [178]. However, knockout of the *IL-12* gene did not significantly affect AdV removal from the host [179]. Therefore, although IL-12 may be important in innate immune activation and the type of subsequent

adaptive immune response to AdV infection, IL-12 does not by itself appear to modulate clearance of AdV.

TNF- α

TNF- α , initially named for its ability to trigger the necrosis and involution of certain tumors [180], is now widely recognized as a mediator of the host response to infection. TNF- α is rapidly produced following either antigen-specific or nonspecific stimulation of the alveolar macrophages, and has been designated as an early alarm response cytokine [142]. TNF- α and IL-1 do not appear to affect polymorphonucleocyte chemotaxis directly, but both of these cytokines are potent inducers for the production of certain CXC chemokines, such as IL-8, by alveolar macrophages, type II epithelial cells and other cells of the airways [85,120]. These chemokines are the major chemoattractants for polymorphonucleocyte recruitment into the lung during pulmonary infection and inflammation [120,181–184]. A study evaluating the expression of TNF- α and IL-1 β after AdV infection of human monocytes (which are nonpermissive for AdV infection) and macrophages [185] showed that the production of both cytokines was enhanced in monocytes. However, in macrophages, a slight enhancement of TNF- α was seen and no IL-1 β was detected. The results suggest that cellular genes might be activated in nonpermissive cells where no viral gene products can be detected. TNF- α signaling through both the p55 and p75 TNF receptors plays important roles in the magnitude of the humoral immune response. Absence of TNF- α or the p55 receptor significantly attenuates the antibody response to AdV [186].

Induction of TNF- α by modified AdV may occur during therapeutic use of the virus. Replication-competent AdVs with various *E1* modifications designed to restrict their replication to tumor cells are being evaluated as oncolytic agents in clinical trials. In mouse models, such oncolytic AdV showed greater dose-dependent hepatotoxicity than *E1*-deleted AdV vectors following intravenous administration. Hepatotoxicity correlated with expression of wild-type *E1A* in the liver with increases in viral DNA levels. This was correlated with rapid induction of TNF- α to high levels and with rapid elevation of serum alanine aminotransferase. Hepatotoxicity was significantly reduced for an AdV with deletions in the region *E1A* (dl01/07) or a virus lacking *E1A*. The results suggest a mechanism for hepatotoxicity involving virus-induced production of local TNF- α release and *E1A*-mediated sensitization of hepatocyte killing [187]. However, AdV hepatotoxicity does not absolutely require AdV gene expression as AdV vectors in which the viral gene expression was completely suppressed caused hepatic toxicity in animal models [145]. Thus, both AdV capsid and AdV proteins are capable of causing cell damage during AdV vector exposure.

Interferons

Interferons are a family of cytokine mediators that influence the quality of cellular immune responses and amplify antigen presentation to specific T cells. IFNs were first discovered by Isaacs and Lindenmann in 1957 by the induction of IFNs in chick cells by influenza virus [188]. In 1967, it was observed that chick embryo fibroblasts inoculated with human AdV produced IFNs [189]. There are three major classes of IFN: IFN- α , IFN- β (type I IFN) and IFN- γ (type II IFN). AdV induces the production of high levels of type I IFN both *in vitro* and *in vivo*.

Type I IFN induction by AdV is the result of two mechanisms involving TLR-dependent and TLR-independent pathways. TLR9 mediates production of IFN by plasmacytoid dendritic cells by AdV and is dependent on MyD88. By contrast, conventional dendritic cells and macrophages produce IFN after detection of cytosolic AdV DNA through TLR-independent pathways. These TLR-independent pathways are triggered when cytosolic

AdV-DNA stimulates the transcription factor IRF3 and mediates the IFN and proinflammatory response [118]. The high levels of type I IFN produced are critical for both the innate and adaptive immune responses against AdV vectors. This was demonstrated when AdV induction of IL-6 and IL-12 and T-cell activation were reduced in mice that were deficient for type I IFN receptors [190].

While type I IFNs are secreted by virus-infected cells, IFN- γ is produced by T cells and NK cells and plays an important role in cell-mediated immunity against a broad spectrum of intracellular pathogens. IFN- γ activates several leukocyte functions, including stimulation of respiratory burst, antigen presentation and priming TNF- α release by macrophages. AdV-7 at multiplicity of infection equal to one induced IFN in human leukocyte cultures [191]. Exposure of cells to IFN induces an antiviral state in which the replication of a wide variety of both DNA and RNA viruses is inhibited [192]. During this antiviral state, a set of cellular genes, termed IFN-stimulated genes, are transcriptionally induced. AdV infection of HeLa cell cultures induced the transcription of these genes, without IFN synthesis or the synthesis of new AdV proteins [193]. However, the E1A gene products specifically suppressed this transcription. The dual effect of AdV on the expression of the IFN-stimulated genes may represent an example of action and evolutionary reaction between virus and host.

Attempts to modify AdV vectors to diminish immune responses

Adenovirus vectors are commonly used for gene therapy. Their use was popularized because, like wild-type AdV, they have weak mutagenicity, the ability to transduce a wide variety of cells [6], and early generations were easily produced. However, early-generation vectors had limited transgene-carrying capacity and were highly immunogenic, which prevented long-term transgene expression and required high-dose administration to provide even short-term transgene expression [194].

The need for further modification of the first-generation AdV vectors (FG-AdV) became apparent after early animal studies, which exhibited local and systemic toxicity. However, lesser, although still important, limitations of FG-AdV as an agent of gene therapy were already known to exist, including adaptive immunogenicity that prevented long-term transgene expression, and a limitation in the transgene size due to viral packaging constraints.

These shortcomings of the FG-AdV vector led to its subsequent modification and the development of second- and third-generation vectors. These vectors had less residual expression of AdV gene products to which the inflammation seen with the first-generation vectors was attributed. As they caused less toxicity in lower animals, trials of high-dose administration in humans were started with a third-generation vector, an *E1*-deleted, *E2A* temperature-sensitive AdV-5. This culminated in the unfortunate death of the 19th patient in the trial.

Perhaps partially in response to this sad event have additional modifications of the vector been performed. Attempts to further reduce residual AdV gene expression resulted in the development of the helper-dependent AdV vector (HD-AdV), also known as the gutless or 'high-capacity' vector [195,196]. HD-AdV vectors have almost all viral DNA deleted except for two elements, the inverted terminal repeats and the packaging domain (ψ) [7]. With the deletion of almost all the viral DNA, the carrying capacity of HD-AdV vectors was substantially increased. Also, in animal models, the toxicity was decreased, while the duration of transgene expression relative to the earlier-generation AdV vectors was enhanced [197–199]. It is thought that there are two phases of AdV toxicity, an initial response caused by the innate immune system, and a second response due to the adaptive immune system [200,201]. In the preceding animal models, it was the second phase of

toxicity that was reduced in the gutless vector-treated animals but the initial phase of toxicity persisted. This suggests that the toxicity of gutless vectors is primarily reduced by removal of the viral coding genes that trigger the adaptive immune response and less owing to a reduction in the innate immune response triggered by viral capsid proteins [202]. However, in one study, the first-generation but not the gutless vector induced acute liver injury in Rag1-deficient mice (lacking mature T and B cells). This suggests that AdV DNA may, in fact, enhance innate immune responses to the virus [196].

However, with the deletion of the viral genes, a production system had to be created that would enable the vectors to replicate. That production system consists of the HD-AdV vector, a producer cell line and a helper virus. One such producer cell line that is used is HEK 293 [203]. It provides the *E1* gene that the HD-AdV vector is missing. The helper virus is an *E1*-deleted FG-AdV. It coinfects with the vector and provides the other viral genes necessary for vector production [204].

Since a helper virus is required for replication, contamination of the HD-AdV vector preparations with helper virus can occur [205], and the contamination can be hazardous. In order to reduce this contamination, several approaches have been tried. The most common approach involves the use of a recombination system such as Cre/*loxP*. Cre is a recombinase that recognizes a specific DNA sequence, the *loxP* site. If Ψ of the helper virus is placed between two *loxP* sites, it can be removed and, thus, prevent packaging of the helper genome into the viral capsid [206].

Other approaches to prevent contamination have included the use of hybrid helper viral systems with either HSV or baculovirus and the helper virus. By combining HSV with a helper virus to make a hybrid helper virus, hybridized helper virus produced during production of the final vector product can be removed. This is done by using known HSV extraction techniques to remove HSV-AdV hybrid virus. The baculovirus system relies on the property of this virus to transduce mammalian cells without replication. Thus, by the use of this baculovirus-AdV hybrid, replication of the helper virus is prevented [207,208]. However, the popularity of the hybrid viral systems has been limited owing to low yield of HD-AdV production and contamination with replication-competent AdV. A different approach relies on size constraints for packaging of the AdV genome. It is based on the use of a protein IX (*pIX*)-deleted helper virus [209]. Deletion of *pIX* results in virions that are heat labile, with capsids that accommodate only 35 kb, of viral DNA. If the viral DNA is larger than 35 kb it cannot be packaged. Therefore, by using a *pIX* helper virus larger than 35 kb, packaging of the helper virus should be limited and contamination prevented. Unfortunately, levels of contamination with this system are no different than with the Cre/*loxP* system. In addition, a kinetic approach has been employed [210]. By inserting a specific DNA sequence, *attB*, between the 5' inverted terminal repeats and the Ψ , the lifecycle of the helper virus is delayed by 14 h. This delay allows helper virus to be harvested before any significant contamination with helper virus has occurred. This strategy is also advantageous because one can still obtain normal titers of helper virus by allowing infection to continue. Additional sophisticated techniques, such as adding 'stuffer DNA' to prevent the formation of undesired contaminating vectors by exploiting packaging limitations of AdV, have been developed [206,211]. Unfortunately, no helper virus removal strategy has yet been successful in eliminating all contamination [13].

Of concern regarding the strategy of modifying AdV vector toxicity by removing larger portions of the internal genome is that the viral particles induce the innate immune response without gene expression. In this regard, it has been demonstrated that viral gene expression is not required in order to induce toxicity. This was demonstrated by using AdV vectors irradiated by UV light with or without psoralen, which is designed to inhibit transcription

but not damage viral surface proteins. AdV treated in this manner induces cytokine production and inflammation in mouse liver, lung and corneal tissue [122,125,212]. Several mechanisms for this directly proinflammatory effect of AdV have been proposed [152,213,214]. It should be noted that the AdV genome may contribute to acute toxicity based on the results previously discussed in Rag1-deficient mice exposed to the so-called gutless vector [196].

Conclusion

Protection from wild-type AdV infection involves both the innate and adaptive immune response. The innate immune system is complex, utilizing mechanical and cellular defenses. Activation of these cellular defenses triggers the release of cytokines and chemokines, which amplify the immune response through the recruitment and activation of other inflammatory cells. These cells then assist with elimination of infectious virus and/or bridge the gap between innate and adaptive immunity.

Much of the acute damage due to AdV infection appears to be owing to deregulation of the immune response. Thus, controlling this response may prove beneficial in suppressing the damage that occurs. This potential therapeutic avenue could be dangerous; however, if it enhanced overall viral replication and increased the infectious burden of the host. This therapeutic goal represents only a slight divergence from the goals of a gene therapist, who does not have to be greatly concerned with replication of the vector.

The current goal of the gene therapist is to create an AdV vector that avoids detection by the immune system and causes no deleterious side effects. Current HD-AdV vectors have been modified in order to achieve such a purpose. They have had much of their viral genome removed in order to avoid precipitating a toxic immune response and to maintain transgene expression. With those deletions come the challenge of maintaining the vector's ability to transduce the host, and producing sufficient quantities of a radically modified vector. Progress has been made with the use of progenitor cells and helper viruses, but despite progress, trials have yet to demonstrate that these modifications have met all the aforementioned goals. A list of ongoing trials is available at [301].

It is now known that further efforts will need to be directed at modulating innate immune events that occur during early stages of vector-cell interaction. This is owing to the fact that direct stimulation of the innate immune system by AdV proteins occurs.

Future perspective

Attempts to lessen the innate immune response continue, with the focus being on three types of approaches: the first being pretreatment of the host, the second relying on modification of the AdV capsid or internal genome, and, finally, modifying the method or route of delivery.

Host pretreatment includes the use of immunosuppressive drugs such as glucocorticoids to globally suppress the immune system, or selective immunosuppression using, for example, with TNF- α blockers. Studies are needed to determine if pre-emptive treatment to avoid or suppress the innate immune response will be successful.

Modifications of the AdV vector include genetic modification and/or removal of AdV DNA, which has already been described in detail in this review, covalent modifications of the AdV capsid using synthetic polymers such as polyethylene glycol, AdV targeting techniques, display of immunoevasive proteins on the AdV capsid and, finally, chimeric AdV vectors.

Results using synthetic polymers, such as polyethylene glycol, have been promising in preliminary studies in mice, and studies in primates are in progress [215–217]. AdV targeting tries to maximize the transduction of the target tissue and minimize the transduction of other tissues by either using tissue-specific promoters or modification of AdV capsid proteins to enhance tissue-specific vector tropism [218]. Display of immunoevasive peptides on the AdV capsid has met with some success in mice, and work in this area is ongoing, including mutating cellular binding sites on the AdV capsid [8,219]. Also, chimeric vectors that display a combination of AdV capsid proteins from multiple AdV serotypes are in development [220–227].

Finally, modification of the method of delivery has been attempted. In the case of hepatic vector delivery, use of a balloon catheter in the vena cava to increase vector contact with the target organ, and decrease dissemination has shown promise in a nonhuman primate model [228].

This is, of course, a brief and incomplete summary of recent techniques that have been used. For a more detailed review of this topic, the reader is referred to the excellent review by Seregin [229].

Additional work will be needed in order to reduce the immune response to AdV or AdV vectors. Perhaps this is finally a chance for the information acquired during wild-type AdV infection studies to be applied to the field of AdV gene therapy. For example, inhibiting interactions of wild-type AdV with integrin receptors by co-administering homologous peptides decreases acute inflammation during experimental keratitis and it is conceivable that similar strategies could be used during gene therapy [125]. This ‘crosspollination’ of ideas, from those studying wild-type infection to those interested in AdV gene therapy, may finally lead to the discovery of techniques suitable for the repair of genetic deficiencies in the laboratory, and also in the clinic.

Bibliography

Papers of special note have been highlighted as

▪ of interest

1. Friedmann T, Roblin R. Gene therapy for human genetic disease? *Science*. 1972; 175(25):949–955. [PubMed: 5061866]
2. Anderson WF. Human gene therapy. *Science*. 1992; 256(5058):808–813. [PubMed: 1589762]
3. Marshall E. Gene therapy death prompts review of adenovirus vector. *Science*. 1999; 286(5448): 2244–2245. [PubMed: 10636774]
4. Assessment of adenoviral vector safety and toxicity. report of the National Institutes of Health Recombinant DNA Advisory Committee. *Hum Gene Ther*. 2002; 13(1):3–13. Describes the NIH response and recommendations following the death of the ornithine transcarbamylase (OTC) gene-therapy research subject. [PubMed: 11779411]
5. Thompson L. Human gene therapy. Harsh lessons, high hopes. *FDA Consum*. 2000; 34(5):19–24. [PubMed: 11590787]
6. Volpers C, Kochanek S. Adenoviral vectors for gene transfer and therapy. *J Gene Med*. 2004; 6(Suppl 1):S164–S171. [PubMed: 14978759]
7. Alba R, Bosch A, Chillon M. Gutless adenovirus: last-generation adenovirus for gene therapy. *Gene Ther*. 2005; 12(Suppl 1):S18–S27. [PubMed: 16231052]
8. Kurachi S, Koizumi N, Sakurai F, et al. Characterization of capsid-modified adenovirus vectors containing heterologous peptides in the fiber knob, protein IX, or hexon. *Gene Ther*. 2007; 14(3): 266–274. [PubMed: 17006548]

9. Schagen FH, Ossevoort M, Toes RE, Hoeben RC. Immune responses against adenoviral vectors and their transgene products: a review of strategies for evasion. *Crit Rev Oncol Hematol*. 2004; 50(1): 51–70. [PubMed: 15094159]
10. Jooss K, Chirmule N. Immunity to adenovirus and adeno-associated viral vectors: implications for gene therapy. *Gene Ther*. 2003; 10(11):955–963. [PubMed: 12756416]
11. Nayak S, Herzog RW. Progress and prospects: immune responses to viral vectors. *Gene Ther*. 2010; 17(3):295–304. [PubMed: 19907498]
12. Rowe WP, Huebner RJ, Gilmore LK, Parrot RH, Ward TG. Isolation of a cytopathic agent from human adenoids undergoing spontaneous degradation in tissue culture. *Proc Soc Exp Biol Med*. 1953; 84:570–573. [PubMed: 13134217]
13. Adenovirus rates remain high. *US Med*. 2000; 36(4):122.
14. Brenner S, Horne RW. A negative staining method for high resolution electron microscopy of viruses. *Biochim Biophys Acta*. 1959; 34:103–110. [PubMed: 13804200]
15. Reddy VS, Natchiar SK, Stewart PL, Nemerow GR. Crystal structure of human adenovirus at 3.5 Å resolution. *Science*. 2010; 329(5995):1071–1075. [PubMed: 20798318]
16. Liu H, Jin L, Koh SB, et al. Atomic structure of human adenovirus by cryo-EM reveals interactions among protein networks. *Science*. 2010; 329(5995):1038–1043. [PubMed: 20798312]
17. Norrby E. The relationship between the soluble antigens and the virion of adenovirus type 3. I Morphological characteristics. *Virology*. 1966; 28(2):236–248. [PubMed: 5932837]
18. De Jong JC, Wermenbol AG, Verweij-Uijterwaal MW, et al. Adenoviruses from human immunodeficiency virus-infected individuals, including two strains that represent new candidate serotypes Ad50 and Ad51 of species B1 and D, respectively. *J Clin Microbiol*. 1999; 37(12):3940–3945. [PubMed: 10565911]
19. Walsh MP, Seto J, Jones MS, Chodosh J, Xu W, Seto D. Computational analysis identifies human adenovirus type 55 as a re-emergent acute respiratory disease pathogen. *J Clin Microbiol*. 2010; 48(3):991–993. [PubMed: 20042633]
20. Hall K, Blair Zajdel ME, Blair GE. Unity and diversity in the human adenoviruses: exploiting alternative entry pathways for gene therapy. *Biochem J*. 2010; 431(3):321–336. [PubMed: 20937040]
21. Wadell G. Molecular epidemiology of human adenoviruses. *Curr Top Microbiol Immunol*. 1984; 110:191–220. [PubMed: 6090058]
22. Gray GC, Goswami PR, Malasig MD, et al. Adult adenovirus infections: loss of orphaned vaccines precipitates military respiratory disease epidemics For the Adenovirus Surveillance Group. *Clin Infect Dis*. 2000; 31(3):663–670. [PubMed: 11017812]
23. Cooper RJ, Hallett R, Tullo AB, Klapper PE. The epidemiology of adenovirus infections in Greater Manchester, UK 1982–96. *Epidemiol Infect*. 2000; 125(2):333–345. [PubMed: 11117957]
24. Booth JL, Metcalf JP. Type-specific induction of interleukin-8 by adenovirus. *Am J Respir Cell Mol Biol*. 1999; 21(4):521–527. [PubMed: 10502562]
25. Alcorn MJ, Booth JL, Coggeshall KM, Metcalf JP. Adenovirus type 7 induces interleukin-8 production via activation of extracellular regulated kinase 1/2. *J Virol*. 2001; 75(14):6450–6459. [PubMed: 11413312]
26. Wanner A, Salathe M, O’Riordan TG. Mucociliary clearance in the airways. *Am J Respir Crit Care Med*. 1996; 154(6 Pt 1):1868–1902. [PubMed: 8970383]
27. Cole AM, Dewan P, Ganz T. Innate antimicrobial activity of nasal secretions. *Infect Immun*. 1999; 67(7):3267–3275. [PubMed: 10377100]
28. Beppu Y, Imamura Y, Tashiro M, Towatari T, Ariga H, Kido H. Human mucus protease inhibitor in airway fluids is a potential defensive compound against infection with influenza A and Sendai viruses. *J Biochem (Tokyo)*. 1997; 121(2):309–316. [PubMed: 9089405]
29. Arnberg N, Edlund K, Kidd AH, Wadell G. Adenovirus type 37 uses sialic acid as a cellular receptor. *J Virol*. 2000; 74(1):42–48. Excellent contemporary review of cellular receptors of adenovirus. [PubMed: 10590089]
30. Arnberg N, Pring-Akerblom P, Wadell G. Adenovirus type 37 uses sialic acid as a cellular receptor on Chang C cells. *J Virol*. 2002; 76(17):8834–8841. [PubMed: 12163603]

31. Arnberg N, Kidd AH, Edlund K, Olfat F, Wadell G. Initial interactions of subgenus D adenoviruses with A549 cellular receptors: sialic acid versus $\alpha(v)$ integrins. *J Virol.* 2000; 74(16): 7691–7693. [PubMed: 10906228]
32. Johansson SM, Arnberg N, Elofsson M, Wadell G, Kihlberg J. Multivalent HSA conjugates of 3'-sialyllactose are potent inhibitors of adenoviral cell attachment and infection. *ChemBioChem.* 2005; 6(2):358–364. [PubMed: 15678425]
33. Kitson C, Angel B, Judd D, et al. The extra- and intracellular barriers to lipid and adenovirus-mediated pulmonary gene transfer in native sheep airway epithelium. *Gene Ther.* 1999; 6(4):534–546. [PubMed: 10476213]
34. Akusjarvi G, Alestrom P, Pettersson M, Lager M, Jornvall H, Pettersson U. The gene for the adenovirus 2 hexon polypeptide. *J Biol Chem.* 1984; 259(22):13976–13979. [PubMed: 6501284]
35. Crawford-Miksza L, Schnurr DP. Analysis of 15 adenovirus hexon proteins reveals the location and structure of seven hypervariable regions containing serotype-specific residues. *J Virol.* 1996; 70(3):1836–1844. [PubMed: 8627708]
36. Toogood CI, Murali R, Burnett RM, Hay RT. The adenovirus type 40 hexon: sequence, predicted structure and relationship to other adenovirus hexons. *J Gen Virol.* 1989; 70(Pt 12):3203–3214. [PubMed: 2481711]
37. Grubb BR, Pickles RJ, Ye H, et al. Inefficient gene transfer by adenovirus vector to cystic fibrosis airway epithelia of mice and humans. *Nature.* 1994; 371(6500):802–806. [PubMed: 7523956]
38. Zabner J, Freimuth P, Puga A, Fabrega A, Welsh MJ. Lack of high affinity fiber receptor activity explains the resistance of ciliated airway epithelia to adenovirus infection. *J Clin Invest.* 1997; 100(5):1144–1149. [PubMed: 9276731]
39. Tecle T, Tripathi S, Hartshorn KL. Review: defensins and cathelicidins in lung immunity. *Innate Immun.* 2010; 16(3):151–159. [PubMed: 20418263]
40. Laube DM, Yim S, Ryan LK, Kisich KO, Diamond G. Antimicrobial peptides in the airway. *Curr Top Microbiol Immunol.* 2006; 306:153–182. [PubMed: 16909921]
41. Sorensen O, Arnljots K, Cowland JB, Bainton DF, Borregaard N. The human antibacterial cathelicidin, hCAP-18, is synthesized in myelocytes and metamyelocytes and localized to specific granules in neutrophils. *Blood.* 1997; 90(7):2796–2803. [PubMed: 9326247]
42. Di Nardo A, Vitiello A, Gallo RL. Cutting edge: mast cell antimicrobial activity is mediated by expression of cathelicidin antimicrobial peptide. *J Immunol.* 2003; 170(5):2274–2278. [PubMed: 12594247]
43. Bals R, Wang X, Zasloff M, Wilson JM. The peptide antibiotic LL-37/hCAP-18 is expressed in epithelia of the human lung where it has broad antimicrobial activity at the airway surface. *Proc Natl Acad Sci USA.* 1998; 95(16):9541–9546. [PubMed: 9689116]
44. Krijgsveld J, Zaat SA, Meeldijk J, et al. Thrombocidins, microbicidal proteins from human blood platelets, are C-terminal deletion products of CXC chemokines. *J Biol Chem.* 2000; 275(27): 20374–20381. [PubMed: 10877842]
45. Ganz T, Lehrer RI. Defensins. *Pharmacol Ther.* 1995; 66(2):191–205. [PubMed: 7667395]
46. Frye M, Bargon J, Dauletbaev N, Weber A, Wagner TO, Gropp R. Expression of human α -defensin 5 (HD5) mRNA in nasal and bronchial epithelial cells. *J Clin Pathol.* 2000; 53(10):770–773. [PubMed: 11064671]
47. Zhao C, Wang I, Lehrer RI. Widespread expression of β -defensin hBD-1 in human secretory glands and epithelial cells. *FEBS Lett.* 1996; 396(2–3):319–322. [PubMed: 8915011]
48. O'Neil DA, Porter EM, Elewaut D, et al. Expression and regulation of the human β -defensins hBD-1 and hBD-2 in intestinal epithelium. *J Immunol.* 1999; 163(12):6718–6724. [PubMed: 10586069]
49. O'Neil DA, Cole SP, Martin-Porter E, et al. Regulation of human β -defensins by gastric epithelial cells in response to infection with *Helicobacter pylori* or stimulation with interleukin-1. *Infect Immun.* 2000; 68(9):5412–5415. [PubMed: 10948173]
50. Harder J, Bartels J, Christophers E, Schroder JM. Isolation and characterization of human β -defensin-3, a novel human inducible peptide antibiotic. *J Biol Chem.* 2001; 276(8):5707–5713. [PubMed: 11085990]

51. Singh PK, Jia HP, Wiles K, et al. Production of β -defensins by human airway epithelia. *Proc Natl Acad Sci USA*. 1998; 95(25):14961–14966. [PubMed: 9843998]
52. Schroder JM, Harder J. Human β -defensin-2. *Int J Biochem Cell Biol*. 1999; 31(6):645–651. [PubMed: 10404637]
53. Garcia JR, Krause A, Schulz S, et al. Human β -defensin 4: a novel inducible peptide with a specific salt-sensitive spectrum of antimicrobial activity. *FASEB J*. 2001; 15(10):1819–1821. [PubMed: 11481241]
54. Harder J, Bartels J, Christophers E, Schroder JM. A peptide antibiotic from human skin. *Nature*. 1997; 387(6636):861. [PubMed: 9202117]
55. Goldman MJ, Anderson GM, Stolzenberg ED, Kari UP, Zasloff M, Wilson JM. Human β -defensin-1 is a salt-sensitive antibiotic in lung that is inactivated in cystic fibrosis. *Cell*. 1997; 88(4):553–560. [PubMed: 9038346]
56. Daher KA, Selsted ME, Lehrer RI. Direct inactivation of viruses by human granulocyte defensins. *J Virol*. 1986; 60(3):1068–1074. [PubMed: 3023659]
57. Smith JG, Nemerow GR. Mechanism of adenovirus neutralization by human α -defensins. *Cell Host Microbe*. 2008; 3(1):11–19. [PubMed: 18191790]
58. Bastian A, Schafer H. Human α -defensin 1 (HNP-1) inhibits adenoviral infection *in vitro*. *Regul Pept*. 2001; 101(1–3):157–161. [PubMed: 11495691]
59. Gropp R, Frye M, Wagner TO, Bargon J. Epithelial defensins impair adenoviral infection: implication for adenovirus-mediated gene therapy. *Hum Gene Ther*. 1999; 10(6):957–964. [PubMed: 10223729]
60. McDermott AM, Redfern RL, Zhang B, Pei Y, Huang L, Proske RJ. Defensin expression by the cornea: multiple signalling pathways mediate IL-1 β stimulation of hBD-2 expression by human corneal epithelial cells. *Invest Ophthalmol Vis Sci*. 2003; 44(5):1859–1865. [PubMed: 12714616]
61. Van Wetering S, Mannesse-Lazeroms SP, Van Sterkenburg MA, Hiemstra PS. Neutrophil defensins stimulate the release of cytokines by airway epithelial cells: modulation by dexamethasone. *Inflamm Res*. 2002; 51(1):8–15. [PubMed: 11852911]
62. Nguyen EK, Nemerow GR, Smith JG. Direct evidence from single-cell analysis that human $\{\alpha\}$ -defensins block adenovirus uncoating to neutralize infection. *J Virol*. 2010; 84(8):4041–4049. [PubMed: 20130047]
63. Smith JG, Silvestry M, Lindert S, Lu W, Nemerow GR, Stewart PL. Insight into the mechanisms of adenovirus capsid disassembly from studies of defensin neutralization. *PLoS Pathog*. 2010; 6(6):E1000959. [PubMed: 20585634]
64. Agerberth B, Gunne H, Odeberg J, Kogner P, Boman HG, Gudmundsson GH. FALL-39, a putative human peptide antibiotic, is cysteine-free and expressed in bone marrow and testis. *Proc Natl Acad Sci USA*. 1995; 92(1):195–199. [PubMed: 7529412]
65. Cowland JB, Johnsen AH, Borregaard N. hCAP-18, a cathelin/pro-bactenecin-like protein of human neutrophil specific granules. *FEBS Lett*. 1995; 368(1):173–176. [PubMed: 7615076]
66. Gudmundsson GH, Agerberth B, Odeberg J, Bergman T, Olsson B, Salcedo R. The human gene *FALL39* and processing of the cathelin precursor to the antibacterial peptide LL-37 in granulocytes. *Eur J Biochem*. 1996; 238(2):325–332. [PubMed: 8681941]
67. Larrick JW, Hirata M, Balint RF, Lee J, Zhong J, Wright SC. Human CAP18: a novel antimicrobial lipopolysaccharide-binding protein. *Infect Immun*. 1995; 63(4):1291–1297. [PubMed: 7890387]
68. Frohm M, Agerberth B, Ahangari G, et al. The expression of the gene coding for the antibacterial peptide LL-37 is induced in human keratinocytes during inflammatory disorders. *J Biol Chem*. 1997; 272(24):15258–15263. [PubMed: 9182550]
69. Frohm Nilsson M, Sandstedt B, Sorensen O, Weber G, Borregaard N, Stahle-Backdahl M. The human cationic antimicrobial protein (hCAP18), a peptide antibiotic, is widely expressed in human squamous epithelia and colocalizes with interleukin-6. *Infect Immun*. 1999; 67(5):2561–2566. [PubMed: 10225921]
70. Guthmiller JM, Vargas KG, Srikantha R, et al. Susceptibilities of oral bacteria and yeast to mammalian cathelicidins. *Antimicrob Agents Chemother*. 2001; 45(11):3216–3219. [PubMed: 11600383]

71. Howell MD, Jones JF, Kisich KO, Streib JE, Gallo RL, Leung DY. Selective killing of vaccinia virus by LL-37: implications for eczema vaccinatum. *J Immunol.* 2004; 172(3):1763–1767. [PubMed: 14734759]
72. Wollenberg A, Engler R. Smallpox, vaccination and adverse reactions to smallpox vaccine. *Curr Opin Allergy Clin Immunol.* 2004; 4(4):271–275. [PubMed: 15238792]
73. Yasin B, Pang M, Turner JS, et al. Evaluation of the inactivation of infectious Herpes simplex virus by host-defense peptides. *Eur J Clin Microbiol Infect Dis.* 2000; 19(3):187–194. [PubMed: 10795591]
74. Agerberth B, Charo J, Werr J, et al. The human antimicrobial and chemotactic peptides LL-37 and α -defensins are expressed by specific lymphocyte and monocyte populations. *Blood.* 2000; 96(9):3086–3093. [PubMed: 11049988]
75. De Y, Chen Q, Schmidt AP, et al. LL-37, the neutrophil granule- and epithelial cell-derived cathelicidin, utilizes formyl peptide receptor-like 1 (FPRL1) as a receptor to chemoattract human peripheral blood neutrophils, monocytes, and T cells. *J Exp Med.* 2000; 192(7):1069–1074. [PubMed: 11015447]
76. Hase K, Eckmann L, Leopard JD, Varki N, Kagnoff MF. Cell differentiation is a key determinant of cathelicidin LL-37/human cationic antimicrobial protein 18 expression by human colon epithelium. *Infect Immun.* 2002; 70(2):953–963. [PubMed: 11796631]
77. Tang YQ, Yeaman MR, Selsted ME. Antimicrobial peptides from human platelets. *Infect Immun.* 2002; 70(12):6524–6533. [PubMed: 12438321]
78. Dhawan VK, Yeaman MR, Bayer AS. Influence of *in vitro* susceptibility phenotype against thrombin-induced platelet microbicidal protein on treatment and prophylaxis outcomes of experimental *Staphylococcus aureus* endocarditis. *J Infect Dis.* 1999; 180(5):1561–1568. [PubMed: 10515816]
79. Mercier RC, Dietz RM, Mazzola JL, Bayer AS, Yeaman MR. Beneficial influence of platelets on antibiotic efficacy in an *in vitro* model of *Staphylococcus aureus*-induced endocarditis. *Antimicrob Agents Chemother.* 2004; 48(7):2551–2557. [PubMed: 15215108]
80. Crouch E, Wright JR. Surfactant proteins A and D and pulmonary host defense. *Annu Rev Physiol.* 2001; 63:521–554. [PubMed: 11181966]
81. Schelenz S, Malhotra R, Sim RB, Holmskov U, Bancroft GJ. Binding of host collectins to the pathogenic yeast *Cryptococcus neoformans*: human surfactant protein D acts as an agglutinin for acapsular yeast cells. *Infect Immun.* 1995; 63(9):3360–3366. [PubMed: 7642263]
82. Madan T, Kishore U, Shah A, et al. Lung surfactant proteins A and D can inhibit specific IgE binding to the allergens of *Aspergillus fumigatus* and block allergen-induced histamine release from human basophils. *Clin Exp Immunol.* 1997; 110(2):241–249. [PubMed: 9367408]
83. Williams MD, Wright JR, March KL, Martin WJ 2nd. Human surfactant protein A enhances attachment of *Pneumocystis carinii* to rat alveolar macrophages. *Am J Respir Cell Mol Biol.* 1996; 14(3):232–238. [PubMed: 8845173]
84. O’Riordan DM, Standing JE, Kwon KY, Chang D, Crouch EC, Limper AH. Surfactant protein D interacts with *Pneumocystis carinii* and mediates organism adherence to alveolar macrophages. *J Clin Invest.* 1995; 95(6):2699–2710. [PubMed: 7769109]
85. Benne CA, Kraaijeveld CA, Van Strijp JA, et al. Interactions of surfactant protein A with influenza A viruses: binding and neutralization. *J Infect Dis.* 1995; 171(2):335–341. [PubMed: 7844369]
86. Hartshorn KL, Crouch EC, White MR, et al. Evidence for a protective role of pulmonary surfactant protein D (SP-D) against influenza A viruses. *J Clin Invest.* 1994; 94(1):311–319. [PubMed: 8040272]
87. Ghildyal R, Hartley C, Varrasso A, et al. Surfactant protein A binds to the fusion glycoprotein of respiratory syncytial virus and neutralizes virion infectivity. *J Infect Dis.* 1999; 180(6):2009–2013. [PubMed: 10558959]
88. Reading PC, Holmskov U, Anders EM. Antiviral activity of bovine collectins against rotaviruses. *J Gen Virol.* 1998; 79(Pt 9):2255–2263. [PubMed: 9747736]
89. Van Iwaarden JF, Van Strijp JA, Ebskamp MJ, Welmers AC, Verhoef J, Van Golde LM. Surfactant protein A is opsonin in phagocytosis of herpes simplex virus type 1 by rat alveolar macrophages. *Am J Physiol.* 1991; 261(2 Pt 1):L204–L209. [PubMed: 1651668]

90. Levine AM, Whitsett JA. Pulmonary collectins and innate host defense of the lung. *Microbes Infect.* 2001; 3(2):161–166. [PubMed: 11251302]
91. Levine AM, Elliott J, Whitsett JA, et al. Surfactant protein-D enhances phagocytosis and pulmonary clearance of respiratory syncytial virus. *Am J Respir Cell Mol Biol.* 2004; 31(2):193–199. [PubMed: 15016617]
92. Levine AM, Whitsett JA, Hartshorn KL, Crouch EC, Korfhagen TR. Surfactant protein D enhances clearance of influenza A virus from the lung *in vivo*. *J Immunol.* 2001; 167(10):5868–5873. [PubMed: 11698462]
93. Harrod KS, Trapnell BC, Otake K, Korfhagen TR, Whitsett JA. SP-A enhances viral clearance and inhibits inflammation after pulmonary adenoviral infection. *Am J Physiol.* 1999; 277(3 Pt 1):L580–L588. [PubMed: 10484466]
94. Van Oud Alblas AB, Van Furth R. Origin, kinetics, and characteristics of pulmonary macrophages in the normal steady state. *J Exp Med.* 1979; 149(6):1504–1518. [PubMed: 448291]
95. Holt PG, Schon-Hegrad MA, Phillips MJ, McMenamin PG. Ia-positive dendritic cells form a tightly meshed network within the human airway epithelium. *Clin Exp Allergy.* 1989; 19(6):597–601. [PubMed: 2688847]
96. Hance AJ. Pulmonary immune cells in health and disease: dendritic cells and Langerhans' cells. *Eur Respir J.* 1993; 6(8):1213–1220. [PubMed: 8224138]
97. Reynolds HY, Newball HH. Analysis of proteins and respiratory cells obtained from human lungs by bronchial lavage. *J Lab Clin Med.* 1974; 84(4):559–573. [PubMed: 4412011]
98. Worgall S, Leopold PL, Wolff G, Ferris B, Van Roijen N, Crystal RG. Role of alveolar macrophages in rapid elimination of adenovirus vectors administered to the epithelial surface of the respiratory tract. *Hum Gene Ther.* 1997; 8(14):1675–1684. [PubMed: 9322870]
99. Zsengeller Z, Otake K, Hossain SA, Berclaz PY, Trapnell BC. Internalization of adenovirus by alveolar macrophages initiates early proinflammatory signaling during acute respiratory tract infection. *J Virol.* 2000; 74(20):9655–9667. [PubMed: 11000238]
100. Greber UF, Willetts M, Webster P, Helenius A. Stepwise dismantling of adenovirus 2 during entry into cells. *Cell.* 1993; 75(3):477–486. [PubMed: 8221887]
101. Huang S, Kamata T, Takada Y, Ruggeri ZM, Nemerow GR. Adenovirus interaction with distinct integrins mediates separate events in cell entry and gene delivery to hematopoietic cells. *J Virol.* 1996; 70(7):4502–4508. [PubMed: 8676475]
102. Wickham TJ, Mathias P, Cheresh DA, Nemerow GR. Integrins and $\alpha_v\beta_3$ $\alpha_v\beta_5$ promote adenovirus internalization but not virus attachment. *Cell.* 1993; 73(2):309–319. [PubMed: 8477447]
103. Duits LA, Ravensbergen B, Rademaker M, Hiemstra PS, Nibbering PH. Expression of β -defensin 1 and 2 mRNA by human monocytes, macrophages and dendritic cells. *Immunology.* 2002; 106(4):517–525. [PubMed: 12153515]
104. Hensley SE, Amalfitano A. Toll-like receptors impact on safety and efficacy of gene transfer vectors. *Mol Ther.* 2007; 15(8):1417–1422. Recent comprehensive review of Toll-like receptor interactions with adenovirus vectors. [PubMed: 17551505]
105. Cerullo V, Seiler MP, Mane V, et al. Toll-like receptor 9 triggers an innate immune response to helper-dependent adenoviral vectors. *Mol Ther.* 2007; 15(2):378–385. [PubMed: 17235317]
106. Robbins CS, Dawe DE, Goncharova SI, et al. Cigarette smoke decreases pulmonary dendritic cells and impacts antiviral immune responsiveness. *Am J Respir Cell Mol Biol.* 2004; 30(2):202–211. [PubMed: 12920055]
107. Esposito DH, Gardner TJ, Schneider E, et al. Outbreak of pneumonia associated with emergent human adenovirus serotype 14 – Southeast Alaska, 2008. *J Infect Dis.* 2010; 202(2):214–222. [PubMed: 20533881]
108. Yoshimura S, Bondeson J, Brennan FM, Foxwell BM, Feldmann M. Antigen presentation by murine dendritic cells is nuclear factor- κ B dependent both *in vitro* and *in vivo*. *Scand J Immunol.* 2003; 58(2):165–172. [PubMed: 12869137]
109. Zhang Y, Chirmule N, Gao GP, et al. Acute cytokine response to systemic adenoviral vectors in mice is mediated by dendritic cells and macrophages. *Mol Ther.* 2001; 3(5 Pt 1):697–707. [PubMed: 11356075]

110. Hirschowitz EA, Weaver JD, Hidalgo GE, Doherty DE. Murine dendritic cells infected with adenovirus vectors show signs of activation. *Gene Ther.* 2000; 7(13):1112–1120. [PubMed: 10918477]
111. Morelli AE, Larregina AT, Ganster RW, et al. Recombinant adenovirus induces maturation of dendritic cells via an NF- κ B-dependent pathway. *J Virol.* 2000; 74(20):9617–9628. [PubMed: 11000234]
112. Philpott NJ, Nociari M, Elkon KB, Falck-Pedersen E. Adenovirus-induced maturation of dendritic cells through a PI3 kinase-mediated TNF- α induction pathway. *Proc Natl Acad Sci USA.* 2004; 101(16):6200–6205. [PubMed: 15071185]
113. Molinier-Frenkel V, Lengagne R, Gaden F, et al. Adenovirus hexon protein is a potent adjuvant for activation of a cellular immune response. *J Virol.* 2002; 76(1):127–135. [PubMed: 11739678]
114. Molinier-Frenkel V, Prevost-Blondel A, Hong SS, et al. The maturation of murine dendritic cells induced by human adenovirus is mediated by the fiber knob domain. *J Biol Chem.* 2003; 278(39):37175–37182. [PubMed: 12855705]
115. Iacobelli-Martinez M, Nemerow GR. Preferential activation of Toll-like receptor nine by CD46-utilizing adenoviruses. *J Virol.* 2007; 81(3):1305–1312. [PubMed: 17108047]
116. Hensley SE, Giles-Davis W, McCoy KC, Weninger W, Ertl HC. Dendritic cell maturation, but not CD8⁺ T cell induction, is dependent on type I IFN signaling during vaccination with adenovirus vectors. *J Immunol.* 2005; 175(9):6032–6041. [PubMed: 16237098]
117. Fejer G, Drechsel L, Liese J, et al. Key role of splenic myeloid DCs in the IFN- α response to adenoviruses *in vivo*. *PLoS Pathog.* 2008; 4(11):E1000208. [PubMed: 19008951]
118. Nociari M, Ocheretina O, Schoggins JW, Falck-Pedersen E. Sensing infection by adenovirus: Toll-like receptor-independent viral DNA recognition signals activation of the interferon regulatory factor 3 master regulator. *J Virol.* 2007; 81(8):4145–4157. [PubMed: 17251283]
119. Cotter MJ, Zaiss AK, Muruve DA. Neutrophils interact with adenovirus vectors via Fc receptors and complement receptor 1. *J Virol.* 2005; 79(23):14622–14631. [PubMed: 16282462]
120. Kunkel SL, Standiford T, Kasahara K, Strieter RM. Interleukin-8 (IL-8): the major neutrophil chemotactic factor in the lung. *Exp Lung Res.* 1991; 17(1):17–23. [PubMed: 2013270]
121. Li Y, Muruve DA, Collins RG, Lee SS, Kubes P. The role of selectins and integrins in adenovirus vector-induced neutrophil recruitment to the liver. *Eur J Immunol.* 2002; 32(12):3443–3452. [PubMed: 12442326]
122. Muruve DA, Barnes MJ, Stillman IE, Libermann TA. Adenoviral gene therapy leads to rapid induction of multiple chemokines and acute neutrophil-dependent hepatic injury *in vivo*. *Hum Gene Ther.* 1999; 10(6):965–976. [PubMed: 10223730]
123. Bals R, Hiemstra PS. Innate immunity in the lung: how epithelial cells fight against respiratory pathogens. *Eur Respir J.* 2004; 23(2):327–333. [PubMed: 14979512]
124. Jimenez-Martinez MC, Mejia H, Linares M, et al. Expression of B7 molecules and TLR-9 on corneal epithelial cells infected with adenovirus: clinico-pathological implications in viral keratoconjunctivitis. *Arch Soc Esp Oftalmol.* 2006; 81(7):391–400. [PubMed: 16888692]
125. Chintakuntlawar AV, Zhou X, Rajaiya J, Chodosh J. Viral capsid is a pathogen-associated molecular pattern in adenovirus keratitis. *PLoS Pathog.* 2010; 6(4):E1000841. [PubMed: 20419141]
126. Stark JM, Amin RS, Trapnell BC. Infection of A549 cells with a recombinant adenovirus vector induces ICAM-1 expression and increased CD-18-dependent adhesion of activated neutrophils. *Hum Gene Ther.* 1996; 7(14):1669–1681. [PubMed: 8886838]
127. Kajon AE, Gigliotti AP, Harrod KS. Acute inflammatory response and remodeling of airway epithelium after subspecies B1 human adenovirus infection of the mouse lower respiratory tract. *J Med Virol.* 2003; 71(2):233–244. [PubMed: 12938198]
128. Bergelson JM, Cunningham JA, Droguett G, et al. Isolation of a common receptor for Coxsackie B viruses and adenoviruses 2 and 5. *Science.* 1997; 275(5304):1320–1323. [PubMed: 9036860]
129. Hong SS, Karayan L, Tournier J, Curiel DT, Boulanger PA. Adenovirus type 5 fiber knob binds to MHC class I α 2 domain at the surface of human epithelial and B lymphoblastoid cells. *EMBO J.* 1997; 16(9):2294–2306. [PubMed: 9171344]

130. Gaggar A, Shayakhmetov DM, Lieber A. CD46 is a cellular receptor for group B adenoviruses. *Nat Med.* 2003; 9(11):1408–1412. [PubMed: 14566335]
131. Segerman A, Atkinson JP, Marttila M, Dennerquist V, Wadell G, Arnberg N. Adenovirus type 11 uses CD46 as a cellular receptor. *J Virol.* 2003; 77(17):9183–9191. [PubMed: 12915534]
132. Varga MJ, Weibull C, Everitt E. Infectious entry pathway of adenovirus type 2. *J Virol.* 1991; 65(11):6061–6070. [PubMed: 1920625]
133. Li E, Brown SL, Stupack DG, Puente XS, Cheresch DA, Nemerow GR. Integrin is $\alpha_v\beta_1$ an adenovirus coreceptor. *J Virol.* 2001; 75(11):5405–5409. [PubMed: 11333925]
134. Walters RW, Grunst T, Bergelson JM, Finberg RW, Welsh MJ, Zabner J. Basolateral localization of fiber receptors limits adenovirus infection from the apical surface of airway epithelia. *J Biol Chem.* 1999; 274(15):10219–10226. [PubMed: 10187807]
135. Pickles RJ, McCarty D, Matsui H, Hart PJ, Randell SH, Boucher RC. Limited entry of adenovirus vectors into well-differentiated airway epithelium is responsible for inefficient gene transfer. *J Virol.* 1998; 72(7):6014–6023. [PubMed: 9621064]
136. Zhang Y, Bergelson JM. Adenovirus receptors. *J Virol.* 2005; 79(19):12125–12131. [PubMed: 16160140]
137. Arnberg N. Adenovirus receptors: implications for tropism, treatment and targeting. *Rev Med Virol.* 2009; 19(3):165–178. [PubMed: 19367611]
138. Booth JL, Coggeshall KM, Gordon BE, Metcalf JP. Adenovirus type 7 induces interleukin-8 in a lung slice model and requires activation of Erk. *J Virol.* 2004; 78(8):4156–4164. [PubMed: 15047831]
139. Steen-Johnsen J, Orstavik I, Attramadal A. Severe illnesses due to adenovirus type 7 in children. *Acta Paediatr Scand.* 1969; 58(2):157–163. [PubMed: 4318009]
140. Schonland M, Strong ML, Wesley A. Fatal adenovirus pneumonia: clinical and pathological features. *S Afr Med J.* 1976; 50(43):1748–1751. [PubMed: 186904]
141. Becroft DM. Histopathology of fatal adenovirus infection of the respiratory tract in young children. *J Clin Pathol.* 1967; 20(4):561–569. [PubMed: 4301496]
142. Zhang P, Summer WR, Bagby GJ, Nelson S. Innate immunity and pulmonary host defense. *Immunol Rev.* 2000; 173:39–51. [PubMed: 10719666]
143. Mistchenko AS, Diez RA, Mariani AL, et al. Cytokines in adenoviral disease in children: association of interleukin-6, interleukin-8, and tumor necrosis factor α levels with clinical outcome. *J Pediatr.* 1994; 124(5 Pt 1):714–720. [PubMed: 8176557]
144. McCoy RD, Davidson BL, Roessler BJ, et al. Pulmonary inflammation induced by incomplete or inactivated adenoviral particles. *Hum Gene Ther.* 1995; 6(12):1553–1560. [PubMed: 8664380]
145. Schnell MA, Zhang Y, Tazelaar J, et al. Activation of innate immunity in nonhuman primates following intraportal administration of adenoviral vectors. *Mol Ther.* 2001; 3(5 Pt 1):708–722. [PubMed: 11356076]
146. Weber SM, Chen JM, Levitz SM. Inhibition of mitogen-activated protein kinase signaling by chloroquine. *J Immunol.* 2002; 168(10):5303–5309. [PubMed: 11994488]
147. Ginsberg HS, Moldawer LL, Sehgal PB, et al. A mouse model for investigating the molecular pathogenesis of adenovirus pneumonia. *Proc Natl Acad Sci USA.* 1991; 88(5):1651–1655. [PubMed: 1848005]
148. Cartmell T, Southgate T, Rees GS, Castro MG, Lowenstein PR, Luheshi GN. Interleukin-1 mediates a rapid inflammatory response after injection of adenoviral vectors into the brain. *J Neurosci.* 1999; 19(4):1517–1523. [PubMed: 9952427]
149. Kolb M, Margetts PJ, Anthony DC, Pitossi F, Gauldie J. Transient expression of IL-1 β induces acute lung injury and chronic repair leading to pulmonary fibrosis. *J Clin Invest.* 2001; 107(12):1529–1536. [PubMed: 11413160]
150. Chang CH, Huang Y, Issekutz AC, Griffith M, Lin KH, Anderson R. Interleukin-1 α released from epithelial cells after adenovirus type 37 infection activates intercellular adhesion molecule 1 expression on human vascular endothelial cells. *J Virol.* 2002; 76(1):427–431. [PubMed: 11739709]
151. Schwarz YA, Amin RS, Stark JM, Trapnell BC, Wilmott RW. Interleukin-1 receptor antagonist inhibits interleukin-8 expression in A549 respiratory epithelial cells infected *in vitro* with a

- replication-deficient recombinant adenovirus vector. *Am J Respir Cell Mol Biol.* 1999; 21(3): 388–394. [PubMed: 10460756]
152. Di Paolo NC, Miao EA, Iwakura Y, et al. Virus binding to a plasma membrane receptor triggers interleukin-1 α -mediated proinflammatory macrophage response *in vivo*. *Immunity.* 2009; 31(1): 110–121. [PubMed: 19576795]
 153. Cruse, JM.; Lewis, RE. *Atlas of Immunology.* CRC Press; FL, USA: 1999. Cytokines; p. 185-206.
 154. Nijsten MW, De Groot ER, Ten Duis HJ, Klasen HJ, Hack CE, Aarden LA. Serum levels of interleukin-6 and acute phase responses. *Lancet.* 1987; 2(8564):921. [PubMed: 2889120]
 155. Kishimoto T, Akira S, Taga T. Interleukin-6 and its receptor: a paradigm for cytokines. *Science.* 1992; 258(5082):593–597. [PubMed: 1411569]
 156. Kawasaki Y, Hosoya M, Katayose M, Suzuki H. Correlation between serum interleukin 6 and C-reactive protein concentrations in patients with adenoviral respiratory infection. *Pediatr Infect Dis J.* 2002; 21(5):370–374. [PubMed: 12150170]
 157. Devergne O, Peuchmaur M, Humbert M, et al. *In vivo* expression of IL-1 β and IL-6 genes during viral infections in human. *Eur Cytokine Netw.* 1991; 2(3):183–194. [PubMed: 1654144]
 158. Ginsberg HS, Lundholm-Beauchamp U, Horswood RL, et al. Role of early region 3 (E3) in pathogenesis of adenovirus disease. *Proc Natl Acad Sci USA.* 1989; 86(10):3823–3827. [PubMed: 2726753]
 159. Boyce JT, Giddens WE Jr, Valerio M. Simian adenoviral pneumonia. *Am J Pathol.* 1978; 91(2): 259–276. [PubMed: 206147]
 160. Pacini DL, Dubovi EJ, Clyde WA Jr. A new animal model for human respiratory tract disease due to adenovirus. *J Infect Dis.* 1984; 150(1):92–97. [PubMed: 6086773]
 161. Prince GA, Porter DD, Jenson AB, Horswood RL, Chanock RM, Ginsberg HS. Pathogenesis of adenovirus type 5 pneumonia in cotton rats (*Sigmodon hispidus*). *J Virol.* 1993; 67(1):101–111. [PubMed: 8380066]
 162. Bruder JT, Kovacs I. Adenovirus infection stimulates the Raf/MAPK signaling pathway and induces interleukin-8 expression. *J Virol.* 1997; 71(1):398–404. [PubMed: 8985363]
 163. Amin R, Wilmott R, Schwarz Y, Trapnell B, Stark J. Replication-deficient adenovirus induces expression of interleukin-8 by airway epithelial cells *in vitro*. *Hum Gene Ther.* 1995; 6(2):145–153. [PubMed: 7734515]
 164. Batshaw M, Raper S, Wilson JM. Study of Adenoviral vector mediated gene transfer in liver in adults with partial ornithine transcarbamylase deficiency (IND 6624) review of data. *J Virol.* 1999:157–178.
 165. Brown RS, Nogrady MB, Spence L, Wiglesworth FW. An outbreak of adenovirus type 7 infection in children in Montreal. *Can Med Assoc J.* 1973; 108(4):434–439. [PubMed: 4347082]
 166. Ladisch S, Lovejoy FH, Hierholzer JC, et al. Extrapulmonary manifestations of adenovirus type 7 pneumonia simulating Reye syndrome and the possible role of an adenovirus toxin. *J Pediatr.* 1979; 95(3):348–355. [PubMed: 224159]
 167. Matsuse T, Hayashi S, Kuwano K, Keunecke H, Jefferies WA, Hogg JC. Latent adenoviral infection in the pathogenesis of chronic airways obstruction. *Am Rev Respir Dis.* 1992; 148:177–184. [PubMed: 1626800]
 168. Elliott WM, Hayashi S, Hogg JC. Immunodetection of adenoviral E1A proteins in human lung tissue. *Am J Respir Cell Mol Biol.* 1995; 12(6):642–648. [PubMed: 7766428]
 169. Meshi B, Vitalis TZ, Ionescu D, et al. Emphysematous lung destruction by cigarette smoke The effects of latent adenoviral infection on the lung inflammatory response. *Am J Respir Cell Mol Biol.* 2002; 26(1):52–57. [PubMed: 11751203]
 170. Keicho N, Elliott WM, Hogg JC, Hayashi S. Adenovirus E1A upregulates interleukin-8 expression induced by endotoxin in pulmonary epithelial cells. *Am J Physiol.* 1997; 272(6 Pt 1):L1046–L1052. [PubMed: 9227502]
 171. Keicho N, Higashimoto Y, Bondy GP, Elliott WM, Hogg JC, Hayashi S. Endotoxin-specific NF- κ B activation in pulmonary epithelial cells harboring adenovirus E1A. *Am J Physiol.* 1999; 277(3 Pt 1):L523–L532. [PubMed: 10484459]

172. Mcmanus TE, Marley AM, Baxter N, et al. Acute and latent adenovirus in COPD. *Respir Med.* 2007; 101(10):2084–2090. [PubMed: 17631991]
173. Chehimi J, Trinchieri G. Interleukin-12: a bridge between innate resistance and adaptive immunity with a role in infection and acquired immunodeficiency. *J Clin Immunol.* 1994; 14(3): 149–161. [PubMed: 7929692]
174. Coutelier JP, Van Broeck J, Wolf SF. Interleukin-12 gene expression after viral infection in the mouse. *J Virol.* 1995; 69(3):1955–1958. [PubMed: 7853541]
175. Miller G, Lahrs S, Pillarisetty VG, Shah AB, Dematteo RP. Adenovirus infection enhances dendritic cell immunostimulatory properties and induces natural killer and T-cell-mediated tumor protection. *Cancer Res.* 2002; 62(18):5260–5266. [PubMed: 12234994]
176. Korst RJ, Mahtabifard A, Yamada R, Crystal RG. Effect of adenovirus gene transfer vectors on the immunologic functions of mouse dendritic cells. *Mol Ther.* 2002; 5(3):307–315. [PubMed: 11863421]
177. Hirschowitz EA, Weaver JD, Hidalgo GE, Doherty DE. Murine dendritic cells infected with adenovirus vectors show signs of activation. *Gene Ther.* 2000; 7(13):1112–1120. [PubMed: 10918477]
178. Peng Y, Falck-Pedersen E, Elkon KB. Variation in adenovirus transgene expression between BALB/c and C57BL/6 mice is associated with differences in interleukin-12 and γ interferon production and NK cell activation. *J Virol.* 2001; 75(10):4540–4550. [PubMed: 11312324]
179. Xing Z, Zganiacz A, Wang J, Divangahi M, Nawaz F. IL-12-independent Th1-type immune responses to respiratory viral infection: requirement of IL-18 for IFN- γ release in the lung but not for the differentiation of viral-reactive Th1-type lymphocytes. *J Immunol.* 2000; 164(5):2575–2584. [PubMed: 10679096]
180. Carswell EA, Old LJ, Kassel RL, Green S, Fiore N, Williamson B. An endotoxin-induced serum factor that causes necrosis of tumors. *Proc Natl Acad Sci USA.* 1975; 72(9):3666–3670. [PubMed: 1103152]
181. Boutten A, Dehoux MS, Seta N, et al. Compartmentalized IL-8 and elastase release within the human lung in unilateral pneumonia. *Am J Respir Crit Care Med.* 1996; 153(1):336–342. [PubMed: 8542140]
182. Standiford TJ, Kunkel SL, Greenberger MJ, Laichalk LL, Strieter RM. Expression and regulation of chemokines in bacterial pneumonia. *J Leukoc Biol.* 1996; 59(1):24–28. [PubMed: 8558063]
183. Greenberger MJ, Strieter RM, Kunkel SL, et al. Neutralization of macrophage inflammatory protein-2 attenuates neutrophil recruitment and bacterial clearance in murine *Klebsiella pneumoniae*. *J Infect Dis.* 1996; 173(1):159–165. [PubMed: 8537653]
184. Gupta S, Feng L, Yoshimura T, Redick J, Fu SM, Rose CE Jr. Intra-alveolar macrophage-inflammatory peptide 2 induces rapid neutrophil localization in the lung. *Am J Respir Cell Mol Biol.* 1996; 15(5):656–663. [PubMed: 8918372]
185. Kristoffersen AK, Sindre H, Mandi Y, Rollag H, Degre M. Effect of adenovirus 2 on cellular gene activation in blood-derived monocytes and macrophages. *APMIS.* 1997; 105(5):402–409. [PubMed: 9201242]
186. Minter RM, Rectenwald JE, Fukuzuka K, et al. TNF- α receptor signaling and IL-10 gene therapy regulate the innate and humoral immune responses to recombinant adenovirus in the lung. *J Immunol.* 2000; 164(1):443–451. [PubMed: 10605041]
187. Engler H, Machemer T, Philopena J, et al. Acute hepatotoxicity of oncolytic adenoviruses in mouse models is associated with expression of wild-type E1a and induction of TNF- α . *Virology.* 2004; 328(1):52–61. [PubMed: 15380358]
188. Isaacs A, Lindenmann J. Virus interference. I The interferon. *Proc R Soc Lond B Biol Sci.* 1957; 147(927):258–267. [PubMed: 13465720]
189. Beladi I, Pusztai R. Interferon-like substance produced in chick fibroblast cells inoculated with human adenoviruses. *Z Naturforsch B.* 1967; 22(2):165–169. [PubMed: 4384712]
190. Zhu J, Huang X, Yang Y. Innate immune response to adenoviral vectors is mediated by both Toll-like receptor-dependent and -independent pathways. *J Virol.* 2007; 81(7):3170–3180. Demonstrates the role of Toll-like receptors in immune responses to adenoviruses in dendritic cell subtypes. [PubMed: 17229689]

191. Pitkaranta A, Hovi T. Induction of interferon in human leukocyte cultures by natural pathogenic respiratory viruses. *J Interferon Res.* 1993; 13(6):423–426. [PubMed: 8151137]
192. Lengyel P. Biochemistry of interferons and their actions. *Annu Rev Biochem.* 1982; 51:251–282. [PubMed: 6180680]
193. Reich N, Pine R, Levy D, Darnell JE Jr. Transcription of interferon-stimulated genes is induced by adenovirus particles but is suppressed by *E1A* gene products. *J Virol.* 1988; 62(1):114–119. [PubMed: 2446013]
194. Gorziglia MI, Lapcevic C, Roy S, et al. Generation of an adenovirus vector lacking E1, e2a, E3, and all of E4 except open reading frame 3. *J Virol.* 1999; 73(7):6048–6055. [PubMed: 10364357]
195. Mitani K, Graham FL, Caskey CT, Kochanek S. Rescue, propagation, and partial purification of a helper virus-dependent adenovirus vector. *Proc Natl Acad Sci USA.* 1995; 92(9):3854–3858. [PubMed: 7731995]
196. Schiedner G, Morral N, Parks RJ, et al. Genomic DNA transfer with a high- capacity adenovirus vector results in improved *in vivo* gene expression and decreased toxicity. *Nat Genet.* 1998; 18(2):180–183. [PubMed: 9462752]
197. Morsy MA, Gu M, Motzel S, et al. An adenoviral vector deleted for all viral coding sequences results in enhanced safety and extended expression of a leptin transgene. *Proc Natl Acad Sci USA.* 1998; 95(14):7866–7871. [PubMed: 9653106]
198. Morral N, O'Neal W, Rice K, et al. Administration of helper-dependent adenoviral vectors and sequential delivery of different vector serotype for long-term liver-directed gene transfer in baboons. *Proc Natl Acad Sci USA.* 1999; 96(22):12816–12821. [PubMed: 10536005]
199. Reddy PS, Sakhuja K, Ganesh S, et al. Sustained human Factor VIII expression in hemophilia A mice following systemic delivery of a gutless adenoviral vector. *Mol Ther.* 2002; 5(1):63–73. [PubMed: 11786047]
200. Gallo-Penn AM, Shirley PS, Andrews JL, et al. Systemic delivery of an adenoviral vector encoding canine factor VIII results in short-term phenotypic correction, inhibitor development, and biphasic liver toxicity in hemophilia A dogs. *Blood.* 2001; 97(1):107–113. [PubMed: 11133749]
201. Worgall S, Wolff G, Falck-Pedersen E, Crystal RG. Innate immune mechanisms dominate elimination of adenoviral vectors following *in vivo* administration. *Hum Gene Ther.* 1997; 8(1):37–44. [PubMed: 8989993]
202. Muruve DA. The innate immune response to adenovirus vectors. *Hum Gene Ther.* 2004; 15(12):1157–1166. [PubMed: 15684693]
203. Graham FL, Smiley J, Russell WC, Nairn R. Characteristics of a human cell line transformed by DNA from human adenovirus type 5. *J Gen Virol.* 1977; 36(1):59–74. [PubMed: 886304]
204. Zhou HS, Zhao T, Rao XM, Beaudet AL. Production of helper-dependent adenovirus vector relies on helper virus structure and complementing. *J Gene Med.* 2002; 4(5):498–509. [PubMed: 12221643]
205. Fisher KJ, Choi H, Burda J, Chen SJ, Wilson JM. Recombinant adenovirus deleted of all viral genes for gene therapy of cystic fibrosis. *Virology.* 1996; 217(1):11–22. [PubMed: 8599194]
206. Parks RJ, Chen L, Anton M, Sankar U, Rudnicki MA, Graham FL. A helper-dependent adenovirus vector system: removal of helper virus by Cre-mediated excision of the viral packaging signal. *Proc Natl Acad Sci USA.* 1996; 93(24):13565–13570. [PubMed: 8942974]
207. Kubo S, Saeki Y, Chiocca EA, Mitani K. An HSV amplicon-based helper system for helper-dependent adenoviral vectors. *Biochem Biophys Res Commun.* 2003; 307(4):826–830. [PubMed: 12878185]
208. Cheshenko N, Krougliak N, Eisensmith RC, Krougliak VA. A novel system for the production of fully deleted adenovirus vectors that does not require helper adenovirus. *Gene Ther.* 2001; 8(11):846–854. [PubMed: 11423932]
209. Sargent KL, Ng P, Eveleigh C, Graham FL, Parks RJ. Development of a size-restricted pIX-deleted helper virus for amplification of helper-dependent adenovirus vectors. *Gene Ther.* 2004; 11(6):504–511. [PubMed: 14999222]

210. Alba R, Hearing P, Bosch A, Chillon M. Differential amplification of adenovirus vectors by flanking the packaging signal with attB/attP-PhiC31 sequences: implications for helper-dependent adenovirus production. *Virology*. 2007; 367(1):51–58. [PubMed: 17560622]
211. Palmer DJ, Ng P. Helper-dependent adenoviral vectors for gene therapy. *Hum Gene Ther*. 2005; 16(1):1–16. [PubMed: 15703484]
212. Wu W, Booth JL, Coggeshall KM, Metcalf JP. Calcium-dependent viral internalization is required for adenovirus type 7 induction of IL-8 protein. *Virology*. 2006; 355(1):18–29. [PubMed: 16890267]
213. Barlan AU, Griffin TM, Mcguire KA, Wiethoff CM. Adenovirus membrane penetration activates the NLRP3 inflammasome. *J Virol*. 2011; 85(1):146–155. [PubMed: 20980503]
214. Muruve DA, Petrilli V, Zaiss AK, et al. The inflammasome recognizes cytosolic microbial and host DNA and triggers an innate immune response. *Nature*. 2008; 452(7183):103–107. [PubMed: 18288107]
215. Croyle MA, Chirmule N, Zhang Y, Wilson JM. PEGylation of E1-deleted adenovirus vectors allows significant gene expression on readministration to liver. *Hum Gene Ther*. 2002; 13(15):1887–1900. [PubMed: 12396620]
216. Croyle MA, Le HT, Linse KD, et al. PEGylated helper-dependent adenoviral vectors: highly efficient vectors with an enhanced safety profile. *Gene Ther*. 2005; 12(7):579–587. [PubMed: 15647765]
217. De Geest B, Snoeys J, Van Linthout S, Lievens J, Collen D. Elimination of innate immune responses and liver inflammation by PEGylation of adenoviral vectors and methylprednisolone. *Hum Gene Ther*. 2005; 16(12):1439–1451. [PubMed: 16390275]
218. Schiedner G, Morral N, Parks RJ, et al. Genomic DNA transfer with a high-capacity adenovirus vector results in improved *in vivo* gene expression and decreased toxicity. *Nat Genet*. 1998; 18:180–183. [PubMed: 9462752]
219. Seregin SS, Aldhamen YA, Appledorn DM, et al. Adenovirus capsid-display of the retro-oriented human complement inhibitor DAF reduces Ad-vector triggered immune responses *in vitro* and *in vivo*. *Blood*. 2010; 116:1669–1677. [PubMed: 20511542]
220. Seregin SS, Amalfitano A. Overcoming pre-existing adenovirus immunity by genetic engineering of adenovirus-based vectors. *Expert Opin Biol Ther*. 2009; 9(12):1521–1531. [PubMed: 19780714]
221. Abbink P, Lemckert AA, Ewald BA, et al. Comparative seroprevalence and immunogenicity of six rare serotype recombinant adenovirus vaccine vectors from subgroups B and D. *J Virol*. 2007; 81(9):4654–4663. [PubMed: 17329340]
222. Wu H, Curiel DT. Fiber-modified adenoviruses for targeted gene therapy. *Methods Mol Biol*. 2008; 434:113–132. [PubMed: 18470642]
223. Nouredini SC, Curiel DT. Genetic targeting strategies for adenovirus. *Mol Pharm*. 2005; 2(5):341–347. [PubMed: 16196486]
224. Youil R, Toner TJ, Su Q, et al. Hexon gene switch strategy for the generation of chimeric recombinant adenovirus. *Hum Gene Ther*. 2002; 13(2):311–320. [PubMed: 11812286]
225. Roberts DM, Nanda A, Havenga MJ, et al. Hexon-chimaeric adenovirus serotype 5 vectors circumvent pre-existing anti-vector immunity. *Nature*. 2006; 441(7090):239–243. [PubMed: 16625206]
226. Liu J, O'Brien KL, Lynch DM, et al. Immune control of an SIV challenge by a T-cell-based vaccine in rhesus monkeys. *Nature*. 2009; 457(7225):87–91. [PubMed: 18997770]
227. McCoy K, Tatsis N, Koriath-Schmitz B, et al. Effect of preexisting immunity to adenovirus human serotype 5 antigens on the immune responses of nonhuman primates to vaccine regimens based on human- or chimpanzee-derived adenovirus vectors. *J Virol*. 2007; 81(12):6594–6604. [PubMed: 17428852]
228. Brunetti-Pierri N, Stapleton GE, Palmer DJ, et al. Pseudo-hydrodynamic delivery of helper-dependent adenoviral vectors into non-human primates for liver-directed gene therapy. *Mol Ther*. 2007; 15(4):732–740. [PubMed: 17285138]
229. Seregin S, Amalfitano A. Improving adenovirus based gene transfer: strategies to accomplish immune evasion. *Viruses*. 2010; 2:2013–2036. Extensive review of the newest attempts to

enhance adenovirus gene-therapy application by modulating the virus and host immune responses.

Website

301. Clinical Trials website. www.clinicaltrials.gov

Executive summary

- Wild-type adenovirus (AdV) infections cause a broad spectrum of illness in both immunocompromised and immunocompetent hosts.
- Innate immune responses to wild-type AdV are important in acute infection and to acute inflammation that occurs during AdV vector gene therapy.
- Modulation of the early-generation AdV vectors may directly or indirectly decrease acute toxicity and improve long-term transgene expression.
- Greater understanding of the innate and adaptive immune response to AdV and AdV vectors may improve the prospects of successful gene therapy.