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Interference with the IL-1-Signaling Pathway Improves the Toxicity Profile of Systemically Applied Adenovirus Vectors

Dmitry M. Shayakhmetov, Zong-Yi Li, Shaoheng Ni, and André Lieber

The safety of gene therapy vectors is a major concern when novel viral or nonviral therapeutics are proposed for applications in humans. Adenovirus (Ad) vectors have been extensively used as efficient gene delivery vehicles in vitro over the last two decades. However, upon i.v. application, they elicit robust innate and inflammatory responses that may be fatal for the host. To date, the primary cytokines and chemokines involved in the initiation of these host responses remain illusive. In this study, we demonstrate that IL-1 is a major mediator involved in the initiation of immediate host responses toward i.v. applied Ad vectors. Using mice in which IL-1 signaling was genetically eliminated (IL-1RI-KO), or wild-type animals for which signaling was blocked by anti-IL-1 Abs, we found that i.v. applied Ad vectors elicited dramatically reduced acute inflammatory responses when compared with control animals. Importantly, the efficiency of Ad gene transfer in vivo was not significantly affected by interfering with IL-1 signaling. Using an in situ hybridization technique, we found that hepatocytes and Kupffer cells trigger IL-1 transcription in liver tissue after i.v. Ad vector administration. We also found that expression of the MIP-2 chemokine gene (which is responsible for recruitment of neutrophils to the liver) depends on IL-1 activation. Our data indicate that immediate innate and inflammatory host responses toward i.v. applied Ad vectors can be pharmacologically controlled through interference with IL-1 signaling pathways. The Journal of Immunology, 2005, 174: 7310–7319.

Adenovirus (Ad) vectors are one of the most commonly used viral vectors in clinical trials in the United States. Their use has been proposed in therapeutics against inborn and acquired human diseases, including cystic fibrosis, cardiovascular disease and cancer (1). More recently, interest in Ad has expanded due to its potential as a vector for vaccination against life threatening infectious agents such as anthrax (2, 3). Cellular and host inflammatory responses to Ad are poorly understood, however. It is currently recognized that manifestation of virus-related systemic toxicity after i.v. administration greatly impairs the safety and efficacy of Ad vectors (4). Intravenously applied Ad is rapidly cleared from the circulation by the liver. The cells of the hepatic reticuloendothelial system, in particular Kupffer cells, are believed to be responsible for the fast clearance of Ad from blood (5–7). Transduction of liver cells is directly associated with a strong innate immune response and systemic toxicity following Ad administration (8–12). Therefore, plasma levels of liver-specific transaminase, alanine aminotransferase (ALT), have been frequently monitored in clinical trials and preclinical studies as a general marker reflecting systemic toxicity of i.v. applied Ad (8, 9, 13–16).

According to the currently accepted model, which is primarily based on in vitro data, Ad infects cells in a two-step process (reviewed in Refs. 17–19). The first step is the binding of the fiber coat protein to the primary cell surface receptor. Most human Ad serotypes (subgroups A–F, but not subgroup B) can use the coxsackievirus and Ad receptor (CAR) as a primary attachment receptor for cell infection (20–23). We and others have recently found that the ubiquitously expressed complement regulatory protein, CD46, is a major subgroup B Ad receptor on human cells (24–26). Following initial attachment, RGD (Arg-Gly-Asp) motifs within the Ad penton base coat protein interact with cellular integrins, facilitating the internalization of attached virus particles into the cell (27). It has been shown that a number of RGD motif-interacting integrins (αvβ1, αvβ5, αvβ6, αvβ8, αβ1, αβ2, αβ2) can serve as secondary receptors, promoting Ad internalization into different cell types in vitro (18, 27–32). Viral interaction with cellular integrins initiates downstream signaling through the activation of two independent but synergistic pathways. The first pathway includes the lipid kinase PI3K, the small G proteins Cdc42 and Rac1, and p38 kinase MAPK (33–36). The second pathway involves signal transduction through focal adhesion kinase, Raf-1, and ERK1/2 protein kinases (34, 37–39). Signal transduction through either of these pathways can induce actin polymerization near the virus attachment site, triggering endocytosis. Importantly, activation of p38 MAPK and ERK1/2 was linked to translocation of NF-κB into the nucleus and to initiation of proinflammatory cytokine and chemokine gene expression (40–43). It has previously been shown that expression of cytokine genes (including TNF-α, IL-6, IFN-γ, IL-1β, and IL-12) and chemokine genes (such as IL-8, MIP-2, IFN-γ-inducible protein 10, RANTES, MIP-1α, MIP-1β, and MCP-1) is significantly up-regulated following Ad infection of different cell types both in vitro and in vivo (33, 40, 41, 44–47). Accumulating data suggest, however, that signaling initiated upon Ad interaction with different primary attachment receptors or cellular integrins may significantly affect the spectrum of cytokines activated in response to Ad infection (48, 49). Moreover, although the mechanism of Ad cell infection...
in vitro is known in great detail, the mechanisms responsible for Ad cell infection in vivo, particularly following intravascular virus application, are still poorly understood. It is currently proposed that after i.v. application, Ad can infect liver cells via two distinct pathways: 1) via the CAR-dependent pathway (20–23), and 2) via a CAR-independent pathway mediated by blood factors (50). The role of each of these pathways of virus cell infection in initiating innate immune and inflammatory host responses is currently unclear. Furthermore, despite numerous studies in animals, and human clinical trials documenting activation of innate immune responses upon i.v. injection of Ad vectors (8–12, 48, 51), the primary mediators involved in the initiation of these host responses remain unidentified. In this study we demonstrate that IL-1 plays a major role in the initiation of innate immune and inflammatory responses associated with intravascular Ad application. We also demonstrate that pharmacologic interference with IL-1 signaling allows for a significant reduction in Ad hepatotoxicity without affecting vector ability to efficiently transduce liver.

**Materials and Methods**

**Ad vectors**

Previously constructed Ad vectors Ad5L and Ad5/35L expressing GFP or β-galactosidase as reporter genes were used (52, 53). Ad5L is a first generation E1- and E3-deleted vector with an unmodified Ad5 capsid. Ad5/35L possesses the Ad35-derived fiber knob domain and the Ad5 fiber shaft domain and capsid. For comparative analyses, identical GFP or β-galactosidase reporter gene expression cassettes were introduced into the E3 region of the Ad genome by homologous recombination in *Escherichia coli* as described earlier (54). For each Ad vector used in this study, at least two independently prepared virus stocks were obtained and characterized by PPU titrating on 293 cells and genome titration by quantitative Southern blot analysis (55). Each produced virus stock was tested for endotoxin contamination using the *Limulus* amebocyte lysate assay, Pyrotell (Associates of Cape Cod). For in vivo experiments, only virus preparations confirmed to be free of endotoxin contamination were used.

**Ad infection in vivo**

All experimental procedures involving animals were conducted in accordance with the institutional guidelines set forth by the University of Washington, Seattle, WA. Knockout (KO) mice for IL-1R type I (B6.129S1.*Il1r1tm1Roml/J, stock no. 003018) and its corresponding control strain B6; 129SF2 (stock no. 101045), as well as C57BL/6J (stock no. 000664) were purchased from The Jackson Laboratory and housed in specific pathogen-free facilities. For analysis of Ad-mediated gene transfer into liver cells, 10^7 Ad particles (corresponds to 5 × 10^7 PFU) in 200 μl of PBS were injected by tail vein infusion. Mice were sacrificed 72 h postvirus infusion and livers were processed for histological analyses. For analysis of Ad genome accumulation in liver tissue, 30 min, 6 and 24 h after Ad vector administration into the tail vein, blood was flushed from the liver by cardiac saline perfusion, livers were harvested, and total DNA was purified and analyzed by Southern blotting as described earlier (49, 55).

To analyze the involvement of TNF-α and IL-1 in the initiation of anti-Ad host responses, C57BL/6J mice were administered with 5 μg/mouse of corresponding functionally blocking rabbit polyclonal Ab 15 min before virus injection. The following Abs, purchased from AbCam were used: anti-IL-1α (code no. ab9724-50), anti-IL-1β (ab9722-50), anti-TNF-α (ab9739-100), and anti-hemagglutinin (HA) epitope tag (ab9110-100) as an irrelevant control Ab. To block IL-1R response, a mixture of both anti-IL-1α and anti-IL-1β Abs was administered into mice. In control settings 10^7 PFU/mL of PBS were injected by tail vein infusion. Mice were sacrificed 72 h postvirus infusion and livers were processed for histological analyses. For analysis of Ad genome accumulation in liver tissue, 30 min, 6 and 24 h after Ad vector administration into the tail vein, blood was flushed from the liver by cardiac saline perfusion, livers were harvested, and total DNA was purified and analyzed by Southern blotting as described earlier (49, 55).

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**Analysis of Ad-Kupffer cell interaction in vivo**

To analyze Ad interactions with Kupffer cells, the Ad vectors were labeled with fluorophore Cy3 (49, 52). Fluorophore-labeled Ads (10^11 particles) were administered by tail vein, and 30 min later livers were flushed with saline via cardiac perfusion, harvested and immediately frozen in OCT compound. Frozen liver sections either remained unstained or were stained with rat anti-mouse F4/80 primary Ab (BD Biosciences) to detect Kupffer cells. Specific binding of primary Abs was visualized with secondary rat Alexa Fluor 488 Ab (Molecular Probes).

**Southern blot analyses**

Isolation of cellular DNA from mouse liver and Southern analysis was performed as described elsewhere (49, 55). A 32P-labeled 8-kb *HindIII* fragment, corresponding to the E2 region of the Ad5 genome, was used for hybridization to specifically detect Ad genomic DNA.

**Analysis of plasma levels of cytokines/chemokines and amino transferases**

To analyze serum levels of proinflammatory cytokines and chemokines, 30 min and 6 and 24 h after i.v. Ad administration, blood samples were collected into heparin-treated Eppendorf tubes, and plasma was obtained and stored at −80°C in small aliquots. To analyze the plasma levels of cytokines/chemokines, a Mouse Inflammatory Cytometric Bead Array (BD Biosciences) was used according to the manufacturer’s protocol. Plasma levels of IL-1β were analyzed using the mouse IL-1β OptEIA ELISA set (BD Biosciences). Plasma samples obtained from at least three mice (for each Ad vector) were analyzed in duplicate. To analyze plasma levels of ALT, the calorimetric ALT detection reagents (Teco Diagnostics) were used according to the manufacturer’s protocol without modifications. Measuring of ALT levels was done in duplicate using plasma samples obtained from at least three mice (per Ad).

**RNase protection assay**

To analyze the mRNA levels of multiple cytokine/chemokine genes in the mouse liver, 10^7 Ad particles were administered into the tail vein, and 30 min and 6 and 24 h postvirus injection, livers were harvested and total RNA was extracted using the RNAqueous-Midi kit (Ambion). Total liver RNA (10 μg) was then hybridized with a mix of 32P-labeled RNA probes. The 32P-labeled RNA probe mix was prepared by in vitro transcription using CK-3 and a custom template set provided by BD Pharmingen. The hybridized RNAs were treated with RNase, using the RNase Protection Assay kit (BD Pharmingen), precipitated and the protected fragments were resolved on vertical sequencing (10% acrylamide) gels. Following electrohoresis, the gels were dried and exposed to x-ray film (Kodak X-Ormat film; Kodak) and PhosphorImager screen (Molecular Dynamics). The signals on the screen were analyzed by PhosphorImager software. The RNase protection assay was performed using RNA samples of at least two to five individual mice per virus. At least two independently prepared virus stocks were used for RNA level analysis and images are represented.

**In situ detection of IL-1α mRNA in liver tissue**

Thirty minutes post i.v. virus application, livers were harvested and OCT compound-embedded thin liver sections were prepared. An in situ RNA hybridization protocol was described in detail (see Ref. 56). Following fixation and dehydration steps, liver sections were hybridized with digoxigenin-labeled (DIG) sense or antisense RNA probe (60°C, overnight), and developed with the DIG Nucleic Acid Detection kit (Roche). To obtain an IL-1-specific probe, the mouse IL-1α encoding sequence was cut out of pCamsil-1α (catalog no. RDB1516; RIKEN Gene Bank, Tsukuba, Japan) and subcloned into pGEMTZ (Promega) in sense or antisense orientation with regards to the T7 promoter. To synthesize DIG-labeled IL-1α-specific probes, a DIG RNA labeling kit (Roche) was used.

**Results**

**Early response to i.v. applied Ad vectors**

To analyze the mediators of the early host response toward i.v. applied Ads we used two previously constructed Ads-based vectors Ad5L and Ad5/35L (49, 52, 53). Although Ad5L can use CAR for efficient cell infection in vitro, Ad5/35L, possessing the Ad35-derived fiber knob domain, infects cells via a CAR-independent pathway (24, 50, 52, 53). After i.v. application in mice, genomes of both vectors accumulated and persisted in liver tissue at similar levels (Fig. 1A) and 72 h postvirus injection hepatocytes expressed comparable levels of GFP (Fig. 1B) or β-galactosidase (Fig. 1C) reporter genes. Both vectors efficiently accumulated in Kupffer cells by 30 min after i.v. application (Fig. 1D).

Changes in RNA levels of >20 different cytokine and chemokine genes were analyzed at different time points after infusion. Based on the kinetics of their expression, genes can be divided into
administration of Ad vectors.

Kupffer cells that accumulated virus (yellow) are indicated by macrophage-specific anti-F4/80 Abs. Counterstained with macrophage-specific anti-F4/80 Abs.

FIGURE 1. Early interactions with liver cells and host responses to i.v. applied Ad vectors. A, Accumulation of Ad in liver tissue. At the indicated time points postvirus injection, total liver DNA was prepared and analyzed by quantitative Southern blot analysis to detect the levels of Ad genomic DNA. B, GFP reporter gene expression in liver tissue. C, Intrahepatic distribution of Cy3-labeled Ad vectors (red) 30 min post i.v. administration and virus accumulation in Kupffer cells, counterstained with macrophage-specific anti-F4/80 Ab (green). Kupffer cells that accumulated virus (yellow) are indicated by arrows. Representative fields are shown. E, Up-regulation of proinflammatory cytokine and chemokine gene transcription in the liver following i.v. Ad administration. At the indicated time points postvirus application, total liver RNA was purified and transcribed into cDNA (Ad5L and Ad5/35L vectors). The magnitude and kinetics of transaminitis was comparable to studies reported earlier (8, 51). Taken together, our studies demonstrate that regardless of whether Ad vectors use CAR-dependent or CAR-independent infection pathways, they cause a similar early host response characterized by the expression and release of proinflammatory cytokines and liver damage upon i.v. injection. Early activation of IL-1 and MIP-2 gene expression in liver tissue and the rapid elevation of IL-1β but not other cytokine levels in plasma may indicate their involvement in the initiation of inflammatory host responses toward i.v. applied Ad vectors.

Initiation of anti-adenovirus host response is mediated by IL-1

Previous studies suggested that TNF-α is likely to be involved in host responses to Ad infection in vitro and in vivo (42, 47, 50, 58, 59, 66–68). To clarify the role of both TNF-α and IL-1 in mediating acute Ad toxicity, we injected mice with Ads that block either TNF-α or IL-1 before virus administration. Control animals were injected with irrelevant species- and isotype-matched anti-HA-tag Abs. Preinjection of anti-TNF-α Abs did not significantly affect activation of IL-1α and IL-1β gene transcription in the liver.
following i.v. injection of Ad5/35L, and resulted in only 1.5-fold reduction of IL-1α gene transcription activation in mice, injected with Ad5L (Fig. 3A). Although we observed a reduction in IL-6 and TNF-α plasma levels 6 h post Ad5L administration in mice, which received anti-TNF-α Ab (Fig. 3B), there was no effect of anti-TNF-α on plasma IL-1β levels. Importantly, ALT levels did not significantly differ in animals preinjected with anti-TNF-α vs control Ab. Taken together these data suggest that TNF-α is not involved in the induction of early inflammatory host responses.

In mice injected with a mixture of functionally blocking anti-IL-1α and anti-IL-1β Abs, the levels of IL-1α gene transcription were reduced 2-fold (Ad5L-injected mice) and 6-fold (Ad5/35L-injected mice) compared with animals injected with control Ab before virus administration (Fig. 3C). Importantly, although the expression of MIP-2 chemokine gene was 40-fold higher than background levels in control animals after i.v. Ad application, it was increased only 2- to 4-fold over the background in animals preinjected with anti-IL-1 Abs. Although at 6 h postvirus application plasma TNF-α levels were not affected by anti-IL-1 Ab injection and IL-6 levels were reduced only after Ad5L but not Ad5/35L vector administration, the hepatotoxicity observed after administration of both viruses was significantly lower in anti-IL-1

**FIGURE 2.** Up-regulation of plasma levels of cytokines and ALT in response to i.v. Ad administration. A, Kinetics of IL-1β, IL-6, and TNF-α plasma levels over the first 6 h after i.v. administration of Ad5L vector in mice. B, Persistence of plasma IL-1β, IL-6, TNF-α and ALT in mice administered with Ad5L or Ad5/35L vectors over the first 24 h post i.v. virus application. Mock-infected animals were administered with saline (Mock). For experiments in (A and B), plasma samples from three individual mice per virus group were collected at the indicated time point. Collected samples were analyzed in duplicate for the levels of indicated cytokines and ALT as described in Materials and Methods.
Involvement of TNF-α in anti-Ad responses

Role of IL-1 in anti-Ad responses

Elimination of IL-1 signaling reduces inflammatory response to i.v. applied Ad vectors

To further elucidate the role of IL-1 signaling in host responses observed following i.v. Ad administration, we used IL-1R type I KO mice. In previous studies, these animals exhibited reduced inflammatory responses upon challenge with hapten or bacteria and no response to IL-1α or IL-1β injection (69). We demonstrate in this study that after i.v. injection of either Ad5L or Ad5/35L, immediate up-regulation of IL-1α and IL-1β was almost abolished in IL-1RI-KO mice compared with control, strain-matched animals (Fig. 4A). Importantly, in contrast to the control group, up-regulation of MIP-2 expression was absent at 30 min postvirus injection, indicating that MIP-2 activation is downstream of and directly depends on IL-1 signaling (Fig. 3C). The markedly reduced level of IL-1αβ mRNA (10-fold less, compared with control animal group) also suggests that IL-1R stimulates its own expression through IL-1R signaling. Consequently, plasma levels of IL-1β detected 30 min post i.v. virus administration were significantly lower in IL-1RI-KO mice compared with control animals (Fig. 4B). IL-6 plasma levels were significantly lower in IL-1RI-KO at 6 h postinfusion of Ad5L and Ad5/35L. TNF-α levels at this time point were reduced in IL-1RI-KO mice in response to Ad5/35L but not to Ad5L vector administration. Importantly, the degree of liver damage after i.v. Ad injection was significantly lower in IL-1RI-KO mice compared with control, strain-matched animals (Fig. 4D). Taken together, these data demonstrate that IL-1 is not only involved in the initiation of anti-Ad host responses, but that signaling through IL-1RI is critical for establishing a proinflammatory milieu (activation of its own transcription and MIP-2, recruiting leukocytes to liver tissue (42, 45, 70, 71) and elevation of inflammatory cytokines and ALT as described in Materials and Methods. * \(p < 0.05\).

FIGURE 3. Involvement of TNF-α and IL-1 in the initiation of innate immune and inflammatory responses toward i.v. applied Ad vectors. A, Up-regulation of gene transcription in liver tissue of mice administered with irrelevant anti-HA-tag Ab (Ad5L and Ad5/35L) and with anti-TNF-α Ab injected animals, compared with the control group (Fig. 3D). Importantly, anti-IL-1 Ab injection did not interfere with Ad-mediated hepatic gene transfer, as assessed based on the level of β-galactosidase transgene expression in the liver (Fig. 3D, bottom right). These data indicate that blocking the IL-1-mediated anti-Ad inflammatory response allows for efficient in vivo gene delivery with markedly reduced vector-associated hepatotoxicity.

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plasma IL-6 levels) that is responsible for the early hepatotoxic side effects upon i.v. Ad vector application.

**IL-1α transcription is up-regulated in hepatocytes and Kupffer cells after i.v. Ad application**

Recent studies demonstrated that Kupffer cell depletion results in a more robust proinflammatory host response to i.v. applied Ad in mice (42) and based on this data, it was suggested that liver sinusoidal endothelial cells are the primary cell type responsible for the systemic inflammatory response initiated after i.v. Ad application. Because our data suggest a major role of IL-1 in the initiation of anti-Ad inflammatory host responses, we visualized IL-1 mRNA in liver tissue in situ to identify cell types involved in these responses. Thirty minutes after Ad5L or Ad5/35L vector injection into C57BL/6J mice, liver sections were analyzed by fluorescent in situ hybridization technique (FISH) using DIG-labeled antisense or sense (as a negative control) mouse IL-1α RNA probes. On confocal microscope images, specific IL-1α mRNA hybridization signals were detectable in hepatocytes and Kupffer cells when antisense IL-1α-specific RNA was used as a probe. No signals were observed on sections hybridized with sense IL-1α RNA (Fig. 5A–D). The conclusion that hepatocytes and Kupffer cells are the cell types that up-regulate IL-1α gene expression was drawn based on the size and the shape of the IL-1α-positive cell nuclei, as well as on the frequency and tissue distribution of these cells in the liver tissue. Staining of cell nuclei on liver sections with ToPro dye and with either Kupffer cell-specific anti-F4/80 or endothelial cell-specific anti-CD31 mAbs revealed characteristic globular shape nuclei for Kupffer cells and spindle shape nuclei for sinusoid endothelial cells (Fig. 5E). However, because the shape of nuclei and tissue distribution for cells expressing IL-1α closely matched those of Kupffer cells, we concluded that Kupffer cells, and not sinusoid endothelial cells, respond to i.v. applied Ad vectors by up-regulating IL-1α gene transcription.

**Discussion**

The liver is the major target of i.v. administered Ad vectors. Thus, the innate arm of the immune system is efficiently activated in this organ following transduction with Ad vectors in vivo via inducing
the expression of numerous cytokines/chemokines and by recruiting immune effector cells (neutrophils, NK cells and monocyte/macrophages) (42, 45, 70, 71). Activation of the immune system leads to rapid elimination of delivered Ad vector genomes (5, 57), and the accompanying inflammation causes acute hepatic toxicity. Notably, acute inflammation is initiated upon interaction between the viral capsid and cellular receptors, and is observed even after i.v. application of helper-dependent Ad vectors, which are devoid of all viral genes (42, 48, 51, 72). Understanding the biology of the early innate host response to Ad vectors is essential to improve the safety and efficacy of Ad-mediated gene therapy. This study is the first to demonstrate a dominant role of IL-1 in the initiation of acute inflammatory host responses upon i.v. Ad administration. Our results are in agreement with earlier studies by Cartmell et al. (73) and Stone et al. (74), who reported that IL-1 mediates a rapid inflammatory response after local injection of Ad vectors into the brain parenchyma.

IL-1 and TNF-α are major mediators of inflammation with similar functions (75). When they are produced at sites of tissue damage or pathogen invasion, these two cytokines act as distress signals to recruit leukocytes by stimulating production of chemotactic factors (e.g., MIP-2, KC, IL-8, and others) and by inducing the expression of adhesion molecules on vascular endothelial cells. In our studies, it was surprising to find that shortly after Ad vector administration IL-1α, but not TNF-α, gene transcription was increased in liver tissue. Moreover, whereas plasma IL-1β levels at 30 min postvirus administration, and IL-6 and TNF-α levels at 6 h postvirus administration were significantly elevated over their background levels, we found no significant up-regulation of transcription of these genes in the liver (Fig. 1E). Two potential explanations may be considered.
to solve this apparent discrepancy between cytokine gene transcription levels in liver tissue and their plasma levels. One is that the cytokines, observed in plasma at 6 h postvirus administration could be released into circulation from activated cells, therefore no transcription of corresponding genes would be necessary to create the observed plasma cytokine levels. The second explanation is that these cytokines were transcribed in and released into the circulation by a different tissue than liver, such as spleen, lung (Ad5 vector genomes have been found in these tissues upon i.v. vector injection) (60–62), or peripheral blood cells (76). Our preliminary studies indicate that in the spleen, the IL-1B gene transcription activation significantly exceeds that of IL-1α, and that TNF-α gene transcription is higher in the spleen than in the liver (D. Shayakhmetov, unpublished observation). This finding is in agreement with earlier studies by the Wilson group (47) reporting that cytokines detected in mouse plasma after i.v. Ad administration (particularly IL-12) were, at least in part, secreted by the spleen. It is noteworthy that Ad vectors (Ad5/35S and Ad5/9S) that are unable to infect hepatocytes or to accumulate within Kupffer cells following i.v. application (due to modified virus capsid structure) induced significantly less IL-1α and MCP-2 gene transcription and no liver damage was observed after their i.v. administration (49), suggesting that the liver, and particularly hepatocytes and Kupffer cells play a major role in mediating Ad toxicity.

Our data demonstrate that IL-1 is immediately expressed in liver tissue after i.v. Ad injection and stimulates expression of the MIP-2 chemokine, which acts as an efficient neutrophil chemoattractant. In earlier studies it was found that CXC chemokines KC and MIP-2 are the primary mediators of neutrophil activation and sequestration in hepatic vasculature in response to a variety of inflammatory mediators (77, 78). Specifically, administration of IL-1α induced significantly higher plasma levels and hepatic mRNA for MIP-2 and KC chemokines, compared with TNF-α (77). Our data indicate that liver MIP-2 gene activation is downstream of and directly depends on the levels of IL-1. Importantly, it was previously found that MIP-2 inactivation with functionally blocking Abs in vivo significantly reduced neutrophil infiltration in liver tissue in response to systemic Ad administration, allowing for the amelioration of Ad-induced hepatotoxicity (45, 70).

Leukocytes, particularly NK cells, can attack Ad transduced hepatocytes and this might account for the elevated ALT levels seen after Ad injection. Furthermore, the proinflammatory cytokine IL-1α can up-regulate functional TLR2 expression in primary-cultured murine hepatocytes and cause apoptosis (79, 80).

Taken together, these data provide a potential explanation for the reduced hepatotoxicity of i.v. applied Ad vectors observed in mice, which lack (IL-1R1-KO) or have blocked (upon preinjection of anti-IL-1 Abs) IL-1 signaling.

Ad interaction with cellular receptors initiates downstream signaling, resulting in activation of ERK1/2 and p38 MAPK (33–39). It has been shown that up-regulation of proinflammatory cytokine and chemokine gene transcription in response to Ad infection in vitro and in vivo was directly associated with ERK and MAPK activation and that inhibition of ERK and MAPK signaling pathways inhibited Ad-induced IFN-γ-inducible protein 10 chemokine expression in mouse liver 1 h following virus administration (39). Furthermore, these authors showed that the levels of IFN-γ-inducible protein 10 and other cytokines and chemokines produced from infected cells in vitro varied dramatically depending on the ability of the Ad to interact with different cellular attachment receptors or integrins. Similarly, in our studies (see Figs. 2B and 3, B and D) we found that IL-6 and TNF-α levels differed upon injection of the CAR-interacting Ad5L vector and the non-CAR interacting Ad5/35L virus. This finding indicates the involvement of different receptors and/or cell types in induction of cytokine expression. Our data also suggest that innate inflammatory host responses can be modulated by restricting or modifying Ad interactions with specific cellular receptors or cell types in vivo. Such modification of virus interactions with host cells should allow for the improvement of the toxicity profile for i.v. applied Ad-based gene therapy vectors.

The cellular receptors and signaling events involved in the transcriptional activation of IL-1 upon Ad infection in vivo remain to be studied. Because both CAR-interacting and non-CAR-interacting Ad vectors induce a similar pattern of early host responses, it seems likely that common cellular receptors are activated upon Ad cell infection in vivo. We have previously shown the involvement of blood factors in uptake of Ad particles into hepatocytes and Kupffer cells upon i.v. application (50, 81). It is plausible to speculate that these CAR-independent pathways might contribute to activation of IL-1 expression in vivo.

Our data provide new insights into the molecular mechanisms involved in host innate immune and inflammatory responses toward systemically applied Ad. Our data also provide a rational approach for pharmacologic management of anti-Ad host responses, allowing for significant improvement of the safety profiles of existing Ad vectors while preserving their gene transfer efficiency.

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Disclosures
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