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Adenovirus sensing by the immune system

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The host immune system developed multiple ways for recognition of viral pathogens. Upon disseminated adenovirus infection, the immune system senses adenovirus invasion from the moment it enters the bloodstream. The soluble blood factors, FX, antibodies, and complement, can bind and activate plethora of host-protective immune responses. Adenovirus binding to the cellular β3 integrin and endosomal membrane rupture trigger activation of IL-1α/IL-1R1 proinflammatory cascade leading to attraction of cytotoxic immune cells to the site of infection. Upon cell entry, adenovirus exposes its DNA genome in the cytoplasm and triggers DNA sensors signaling. Even when inside the nucleus, the specialized cellular machinery that recognizes the double-strand DNA breaks become activated and triggers viral DNA replication arrest. Thus, the host employs very diverse mechanisms to prevent viral dissemination.

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Introduction

Prompt recognition of a viral invasion and timely activation of antiviral mechanisms are crucial survival factors for the host. The diversity of viruses and the continuous threat they pose, forced hosts to develop multiple ways of recognizing 'non-self' virus determinants. The pathogen-sensing mechanisms in the host target every step of viral infection ensuring detection and elimination of the pathogen by the immune system. Activation of the immune system by Adenovirus (Ad) triggers multiple defense mechanisms aimed at clearing the viral pathogen. The first steps of the immune activation include: (i) activation of a systemic pro-inflammatory state, (ii) attracting cytotoxic immune cell populations to the sites of infection to eliminate virus-containing cells, and (iii) alarming neighboring uninfected cells of viral infection. The systemic proinflammatory state is achieved by the release of proinflammatory cytokines IL-6, tumor necrosis factor alpha (TNFα), and IL-1β into the bloodstream [1]. Macrophages (MF) in different organs represent the first cellular line of defense that traps incoming Ad, thus withdrawing it from the bloodstream [2,3•,4–6]. Next, infected MF release different cytokines and chemokines that function as chemoattractants for the cytotoxic immune cells. The IL-1α/IL-1R1 pathway is critical for the activation of incoming cytotoxic cells to eliminate infected cells and prevent virus spread [7]. To alarm neighboring non-infected cell of a viral infection, Ad infected cells release type I interferons (IFN), IFNα and IFNβ [8]. These cytokines activate the expression of numerous effector antiviral genes in surrounding cells, preparing uninfected cells to enter an antiviral state. Given the complexity of the immune response to viral infection it is quite difficult to untangle the signaling pathways in different cell populations in the host and determine particular roles of pathways in viral clearance. This review focuses on the recent data of the mechanisms that hosts employ for sensing Ad vector and signaling pathways activated by Ad vector infection.

Blood factor signaling

Sensing Ad vectors by the host occurs at all stages of viral invasion starting from the moment it enters the bloodstream. Upon intravascular delivery, the Ad vector interacts with multiple blood factors, including coagulation factor X (FX), neutralizing and natural antibody (Ab), and complement components C3 and C4 [4,9–11]. The host machinery detects the interactions of Ad with blood factors and activates the immune system. Binding of FX to the Ad hexon, one of the major capsid proteins, results in nuclear factor kappa B (NFκB)-dependent transcriptional activation of IL-1β cytokine in the spleen [9]. The mechanism of detection of Ad-FX complex *in vivo* requires toll-like receptor 4 (TLR4) signaling and functional activity of TLR4 adaptors MyD88, TRAF6, and TRIF. In support of this, a mutant Ad5 vector containing a single mutation in the hexon protein T425A that ablates binding to the FX fails to elicit IL-1β transcription *in vivo* [9]. Additionally, Ad subtypes that are naturally incapable of FX binding elicit a diminished multitude of cytokines compared to Ad subtypes that bind the FX [9]. The TLR4 signaling was also shown to be important for the ability to attract and retain

polymorphonuclear leukocytes (PMNs) in the marginal zone of the spleen. As a result, mice deficient in TLR4 failed to clear the virus [9]. In addition, other blood soluble factors besides FX have been shown to play a role in sensing Ad. McEwan *et al.* showed that the tripartite motif containing 21(TRIM21), an intracellular antibody receptor, can recognize Ab-Ad complexes and trigger activity [11]. TRIM21 was able to recognize intracellular Ab and activate the synthesis of an unanchored Lys63 polyubiqitin chain, which, in turn, activated, Activator protein 1 (AP1), and interferon regulating factor (IRF) signaling pathways. As a result, TRIM21-deficient MEFs had significantly (8-1000 fold) lower expression of a variety of pro-inflammatory cytokines and chemokines (IL-6, CXCL10, CCL2, CCL4, TNFα, and IFNβ) in response to treatment with Ad-Ab complex. The activation of signaling pathways by Abs in the cytosol was independent of FcR or pattern recognition receptors (PRRs), but dependent on transforming growth factor beta-activated kinase 1 (TAK1) and signaling [11]. Interestingly, no difference was found in activating abilities between IgG or IgM. Moreover, transfection of Abs bound to the latex beads was also able to activate signaling in MEFs [11]. Another report implicating a blood factor, complement component C3, in Ad sensing came from the same group. Tam *et al.* showed that C3 in complex with Ad can be detected in the cytosol by the unknown cytoplasmic receptor [12•]. The intracellular sensing of the complement activated inflammatory cascades dependent on mitochondrial activating signaling protein (MAVS) and TRAF signaling. Interestingly, cells lacking complement receptors on the surface were able to activate the signaling cascades only after C3-Ad complex is located in the cytoplasm, whereas cells that have activating complement receptors CR1, CR3, or CR4 were able to sense the complex on the cellular surface and activate signaling [12•]. The studies implicating the intracellular sensing of complement components and antibodies in the activation of the immune system have been performed exclusively in tissue culture and their applicability in the *in vivo* settings require further elucidation. In summary, the blood components FX, C3, and natural antibodies represent very ancient pathogen detection system and their relocalization into the cytosol triggers a non-specific systemic proinflammatory response.

Macrophages signaling

The next step of pathogen recognition occurs when the virus attaches to the receptor on the cellular surface and enters the cell. The fiber protein of the Ad capsid mediates the primary receptor attachment of the virus. The very distal fiber domain, fiber knob, binds to the primary species-specific receptor activating the virus entry to the cell [13]. The secondary attachment is mediated by the RGD loops located on the penton protein of Ad capsid. The RGD motif on the penton functions as a ligand for α3β1, α5β1, αvβ1, αvβ3, and αvβ5 integrins on the surface of the cell [14]. In the organs, residential MFs are the first line of the cells that interacts with the incoming Ad vector. In the spleen $MARCO^+$ MΦs trap the virus from the bloodstream and release IL-1α thus activating IL-1R1 dependent innate immune response [15]. The engagement of β3 integrins during Ad entry was found to be critical for IL-1α expression. The mice deficient in β3 integrin did not activate full immune response upon Ad vector injection, and reciprocally, Ad vector with deleted RGD motif was unable to activate innate immune response [16]. The Ad ts1 mutant that binds to the β3 integrins but is unable to rupture the endosome was also very weak innate immune system inducer [15]. However, the ts1 mutant was able to induce the expression of IL-1α mRNA, but failed to produce functionally active IL-1α. These data suggest that engagement of β3 integrin on the cellular surface triggers activation of IL-1α mRNA transcription, but for cytokine production the second signal, such as an endosomal rupture is required. Furthermore, IL-1α signaling through the IL-1R1 and activation of expression CXCL1 and CXCL2 chemokines leads to attraction of PMNs to the sites of infection [2,7]. Interestingly, that complement component C3 cooperated for the recruitment of PMNs to the spleen. Treatment of mice with complement inhibitory proteins resulted in partial redistribution of PMNs from the spleen marginal zone, while the same treatment of IL-1α-deficient mice completely ablated PMNs localization in the marginal zone. In summary, in the spleen the activation of the key IL-1α/IL-1R1 proinflammatory pathway is dependent on β3 integrin-Ad penton RGD interaction, as well as on the endosomal membrane rupture [2]. The IL-1α signaling cooperates with complement C3 for attracting and retaining PMNs in the spleen marginal zone that kill and eliminate infected MFs.

In the liver the first cells that encounter the incoming vector are Kupffer cells, residential liver MΦs. Kupffer cells trap the virus from the bloodstream and in the attempt to eliminate viral burden by undergoing a suicidal necrotic cell death [17,18]. The Kupffer cell death occurs within the first hour post Ad vector injection and does not require signaling of any known mediators of necrotic cell death [3•,19]. However, Kupffer cells in mice deficient in IRF3 were unable to trigger the necrotic cells death, subsequently leading to significantly lower amount of proinflammatory cytokines and chemokines in the plasma and higher viral load at 24 h post injection in $IRF3^{-/-}$ mice than in wt animals [3•]. The absence of the MΦ death was also observed in the wt mice injected with the ts1 mutant of Ad that is incapable of escaping the endosomes. Interestingly, the deficiency in the upstream activators of IRF3 (MAVS, stimulator of interferon genes (STING), and DNA-dependent activator of interferon-regulatory factors (DAI)) did not prevent Kupffer cell death suggesting an alternative mechanism for sensing