Influence of host proteins on the innate immune response to human adenoviruses in human phagocytes

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Human adenoviruses (HAdV) cause a broad spectrum of clinical diseases in immunocompromised and –competent patients and are also versatile tools for gene transfer and vaccination. Pre-existing humoral immunity may be in part responsible for the adverse responses towards AdV vectors seen in several clinical vaccine trials. Furthermore, different host proteins like coagulation factor X (FX) or immunoglobulin G (IgG) bind HAdV and may exacerbate the pro-inflammatory response. Pre-clinical risk assessment is often done in mice, albeit there are multiple differences between human and mice in the interaction with HAdV. The binding of mouse FX to HAdV activates a pro-inflammatory response in mouse via Toll-like receptor 4 (TLR4). Because AdV infection in immunocompromised individuals are a severe threat and can reach concentrations of 10^9 viral particles/mL blood, a human FX-HAdV-TLR4 would play a crucial role in systemic inflammation. In another clinical relevant scenario, human serum containing HAdV-C5-specific IgG increases significantly secretion of the pro-inflammatory cytokine IL-1β, a surrogate marker for inflammasome formation, but by unknown mechanism. In this regard, I participated in two research studies and contributed to one review article. In this resume, I will focus on the two research studies.

1. **Human coagulation factor X-adenovirus type 5 complexes poorly stimulate an innate immune response in human mononuclear phagocytes**

   Karsten Eichholz, Franck Mennechet, Eric J.Kremer

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Here, I investigated a potential role of FX and TLR4 in the innate response to HAdV-C5 by using only human components. Because pre-existing humoral immunity reshapes innate immunity against HAdV-C5, we performed our experiments in a paradigm of primary and secondary infection (Fig. 1A). We assayed the effect of HAdV-C5 complexed with FX and/or HAdV-specific IgG on different levels of the TLR4 pathway (Fig. 1B). We found that there is no detectable FX-HAdV-TLR4 axis in human and FX did not affect the innate immune response elevated by immune complexed HAdV-C5 (IC-HAdV-C5) in human phagocytes.

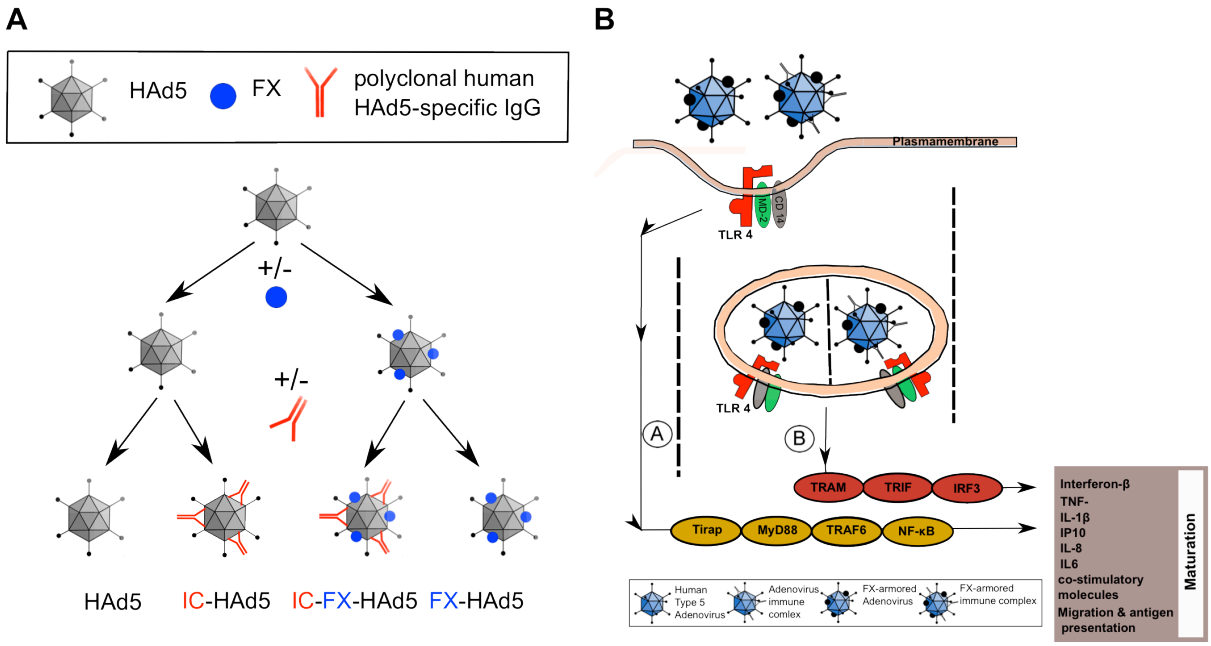

Figure 1: Study design to understand if coagulation factor X (FX)-complexed HAdV-C5 induces TLR4. Four different stimuli were formed by sequential incubation of HAdV-C5 with FX and/or HAdV-C5 specific IgG (A). Induction/interaction of the TLR4 pathway by/with the different stimuli with was assessed by virus binding to the cell surface, TLR4-internalization, secretion/mRNA expression of Nf-kB and IRF3 dependent proinflammatory cytokines and interferons, and cell surface expression of dendritic cell maturation marker and co-stimulatory molecules (B).

2. Immune-complexed adenoviruses induce pyroptotic dendritic cells death by activating vesicular and cytoplasmic DNA sensors

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Here, we addressed the underlying mechanism of IC-HAdV-C5-induced inflammation leading to increased IL-1β secretion in human primary phagocytes. A central step in the onset of inflammation is inflammasome formation, a multiprotein platform in the cytosol. Inflammasome sensors like the DNA sensor Absent in Melanoma 2 (AIM2) surveil the cytosol for microbial DNA and trigger inflammasome assembly upon DNA binding. Another inflammasome sensor, the Nod-like receptor NLRP3 is involved in inflammasome assembly upon endosomal escape of HAdV-C5 alone. A priori, one would expect that IgG opsonization of HAdV-C5 prevents escape into the cytosol. We investigated IC-HAdV-C5 trafficking after Fcγ-receptor-mediated endocytosis and cellular events leading to inflammasome assembly by using RNAi, ELISA, microscopy, flow cytometry and HAdV mutants defective in endosmal escape (Fig. 2). We found that IC-HAdV-C5 induces inflammasome formation in monocyte-derived dendritic cells and this is dependent on capsid protein VI-mediated endosomal escape and activation of cytosolic AIM2 (Fig. 2). This study has implications for individuals suffering from HAdV-disseminated disease where elevated levels of IL-1ß, type-specific antibodies and high titers of virus in the blood can be found.

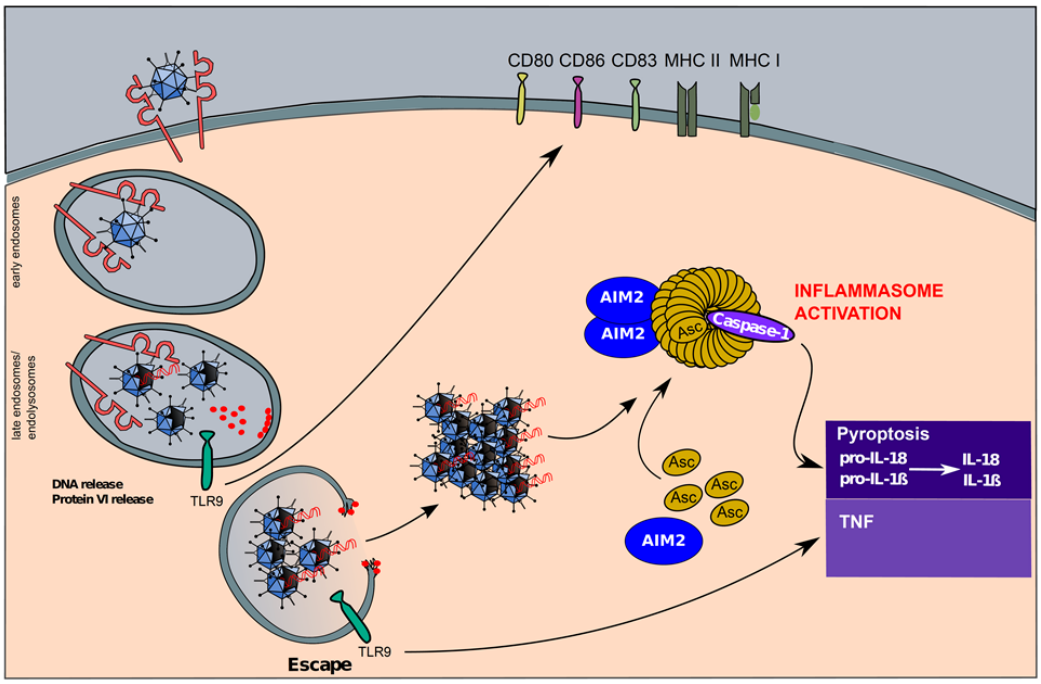

Figure 2: Immune complexed-HAdV-C5 (IC-HAdV-C5) induce the AIM2 inflammasome after endosomal escape. IC-HAdV-C5 are internalized via Fcγ receptor-mediated endocytosis (upper left) and partially disassemble during endosomal maturation and leads to the release of viral genome and capsid protein VI (middle left). While the viral genome induces TLR9, protein VI can lyse the endolysosomal membrane. In turn, IC-HAdV-C5 aggregates into particles of several micrometers in diameter intracellular and induce the AIM2 inflammasome assembly.

Our findings help us to better understand the differences in preclinical testing in mice and clinical use in humans and how pre-existing immunity shapes the innate immune response to HAdV to improve treatment for HAdV diseases and HAdV vector effectiveness.