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Male Syrian hamsters are more susceptible to intravenous infection with species C human adenoviruses than are females

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ABSTRACT

Recently, increasing attention has been focused on the influence of sex on the course of infectious diseases. Thus far, the best-documented examples point toward an immune-mediated mechanism: the generally stronger immune response in females can result in a faster clearance of the pathogen or, conversely, a more severe immune-mediated pathology. Here, we report that human species C adenoviruses replicate more and cause more pathology in male Syrian hamsters than in females. We also show that this sex disparity is not caused by a stronger immune response to the infection by the female hamsters. Rather, the liver of male hamsters is more susceptible to adenovirus infection: after intravenous injection, more hepatocytes become infected in male animals than in females. We hypothesize that Kupffer cells (hepatic tissue macrophages) of female animals are more active in sequestering circulating virions, and thus protect hepatocytes more efficiently than those of males.

1. Introduction

Human adenoviruses (HAdV) are ubiquitous viruses with a double-stranded DNA genome of ca. 36 kb, and a non-enveloped icosahedral capsid. Nearly all humans are infected with one or more HAdV types at an early age. Approximately 5% of "common cold" infections in children are due to HAdV, as are a significant proportion of gastrointestinal infections (Wold and Ison, 2013). While HAdV infections are usually mild and self-resolving in adults, some HAdV types can cause serious infection in some cases, such as acute respiratory distress in military personnel and epidemic keratoconjunctivitis, a serious eye infection. However, the most significant disease caused by HAdV is the often deadly disseminated infection of immunosuppressed transplant patients, especially pediatric patients. The defective immune system of these children cannot eliminate the virus which can spread throughout the body and cause multi-organ disease, with case fatality rates reported in the literature between 10% and 50% (Echavarria, 2008; Ison, 2006; Ison and Hayden, 2016; Lion, 2014; Stercz et al., 2012; Wold and Ison, 2013). Currently, there is no FDA-licensed treatment for disseminated HAdV infection, although case reports and clinical trial data suggest that cidofovir, brincidofovir, and ganciclovir may be active against the virus (Lenaerts and Naesens, 2006; Lindemans et al., 2010; Matthes-Martin et al., 2013; Wold and Toth, 2015).

To research HAdV pathogenesis in vivo and to aid the development of anti-HAdV drugs, our laboratory pioneered the development and characterization of a Syrian hamster model (reviewed in (Wold and Toth, 2012)). The scientific premise of this model is that HAdVs replicate in these animals and that the infection reproduces the pathology seen in humans. Syrian hamsters are one of the two rodent species (the other is the cotton rat) permitting species human mastadenovirus C (HAdV-C) types 1, 2, 5, and 6 infection and replication. Using this model, it became possible to investigate the mechanism and efficacy of oncolytic AdVs, carry out controlled in vivo experiments to investigate the pathogenesis of HAdVs, and test the efficacy of anti-adenoviral compounds (Wold and Toth, 2012, 2015).

We showed that HAdV-C5 and C6 injected intravenously (i.v.) into Syrian hamsters replicates in multiple organs, most prominently in the liver (Lichtenstein et al., 2009; Tollefson et al., 2017; Ying et al., 2009). With immunocompetent hamsters, the immune response efficiently eliminates the virus, and no infectious HAdV can be recovered at 7–10 days post infection (p.i.). Thus, similarly to humans, an intact immune system is essential to limit replication and pathogenesis. Similarly to immunocompromised humans, Syrian hamsters immunosuppressed (by cyclophosphamide [CP]), cannot eliminate the infection, and the virus burden in the liver can reach 10^{10} 50% tissue culture infectious dose ($TCID_{50}$) per gram of tissue (Thomas et al., 2006). Just like in immunocompromised patients, HAdV causes fulminant hepatitis in these animals. Hamsters develop quantifiable pathology (mortality, weight loss, elevated serum transaminase levels, microscopic pathology) (Lichtenstein et al., 2009; Ying et al., 2009). Contrary to mice, in which

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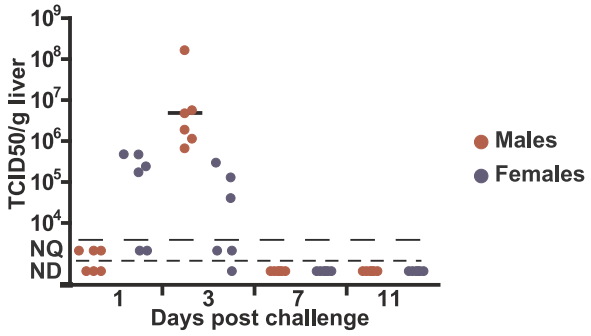

Fig. 1. After HAdV-C6 infection, immunocompetent male hamsters have higher virus burden in their liver than females. Hamsters were infected i.v. with 6×10^{10} PFU of HAdV-C6 per kg. The animals were sacrificed at the indicated time points, and virus was extracted from the liver and quantified by $TCID_{50}$ assay on cultured HEK-293 cells. In this and subsequent figures, symbols represent data from individual animals, and the horizontal bar depicts the geometric mean. NQ: not quantifiable; ND: not detectable.

HAdV replicates very poorly, the liver damage observed in hamsters is due to infection and lysis of hepatocytes rather than a cytokine storm released by dying Kupffer cells (Tollefson et al., 2017). Thus, with hamsters, the i.v. route of infection results in a disease akin to the one seen with immunocompromised patients with advanced multi-organ HAdV infections. Species C HAdVs delivered through the nose cause pneumonia in hamsters, resulting in rapid weight loss; the virus replicates in the ciliated bronchial epithelial cells (Tollefson et al., 2017). This route of infection can be used to model respiratory infections in humans.

As described above, the immune response is essential to fight infection. We showed that the type I IFN response is a crucial component of the innate immune response to HAdV infection; upon infecting STAT2 KO hamsters with HAdV-C5 i.v., we observed that these hamsters were much more susceptible to HAdV infection than wild-type (wt) ones, and that the HAdV-C5 burden is 1000-fold higher in the STAT2 KO animals than in wild type (Toth et al., 2015).

In our experiments with HAdV-infected hamsters we observed anecdotal evidence that there is a sex-specific difference in the pathogenicity of species C HAdVs in hamsters. We now report that after i.v. infection, HAdV-C6 indeed replicates better and is more pathogenic in male animals than in females.

2. Materials and methods

2.1. Cells and viruses

The HEK-293 human embryonic kidney cell line was obtained from Microbix (Toronto, ON), while the A549 human lung adenocarcinoma cell line was purchased from ATCC (Manassas, VA). Both cell lines were cultured in DMEM supplemented with 10% fetal bovine serum. Human HAdV-C6 (VR-6; Tonsil 99) was purchased from ATCC and cultured and purified as described in (Tollefson et al., 2007). AdKD3E3-Luc is an E1- and E3-deleted replication defective (RD) HAdV vector based on HAdV-C5, which expresses firefly luciferase from the CMV promoter. The titer of the virus stocks was determined by plaque assay (Tollefson et al., 2007).

2.2. Syrian hamsters

Syrian hamsters (*Mesocricetus auratus*) were purchased from Envigo (Harlan, Indianapolis, IN) at approximately 80 g body weight. All studies were approved by the Institutional Animal Care and Use Committee of Saint Louis University and were conducted according to federal and institutional regulations.

2.3. Infection of hamsters with adenovirus

In some experiments, the hamsters were immunosuppressed using cyclophosphamide (CP) (Toth et al., 2008). CP was administered intraperitoneally (i.p.) twice weekly for the duration of the study, starting with an induction dose of 140 mg/kg, and then at a dose of 100 mg/kg for all subsequent injections. For i.v. infection, HAdV-C6 was injected i.v. (via the jugular vein) in 200 µl of PBS after anesthetizing the animals with ketamine/xylazine, while for respiratory infection, the virus was pipetted into the nostrils of isoflurane-anesthetized hamsters in 100 µl of PBS.

All hamsters were observed and weighed daily. Animals were sacrificed at different time points depending on the study, or when their health status necessitated the sacrifice. At necropsy, serum and select organs were collected. Virus was extracted from the liver, lung, and kidney and was quantified by $TCID_{50}$ assay. Briefly, parts of the organs (the right lobe of the liver, the left lobe of the lung, and the left kidney) were homogenized in a bead-beater, the cells were disrupted by sonication and repeated freeze-thaw cycles, and the virus content was determined by $TCID_{50}$ assay in HEK-293 cells (Toth et al., 2008). Serum was assayed for liver-specific analytes and for neutralizing antibodies (Toth et al., 2015). Liver histopathology was scored on a scale of 0 (no lesions) to 4 (marked lesions). Immunohistochemical staining was performed by Deborah Berry and the team at Histopathology & Tissue Shared Resource (HTSR) (via Science Exchange), using 1:1000 dilution of the Adenovirus Ab-4 (4D2) (Lab Vision, Fremont, CA) antibody to stain for the HAdV fiber protein. The number of fiber positive cells was quantified by counting stained cells in three randomly chosen view fields at 60X magnification.

2.4. Determining serum neutralizing antibody levels

Anti HAdV-C6 neutralizing antibodies (NAb) in the serum were quantified as described in (Toth et al., 2015). Briefly, serum samples were inactivated by heat treatment at 56 °C for 30 min. One hundred PFU of HAdV-C6 was incubated with twofold serial dilutions of serum samples at 37 °C for 1 h. After that, A549 cells were infected with the virus-serum mixture, and the NAb titer was calculated as the reciprocal dilution causing 50% inhibition of viral cytopathic effect.

2.5. Determining the relative mRNA abundance for immune-related genes using reverse transcriptase-quantitative polymerase chain reaction (RT-qPCR)

Total RNA from liver was extracted from each hamster by homogenizing a fraction of collected tissue samples in RLT lysis buffer (Qiagen, Valencia, CA) and then extracting the RNA using the RNeasy mini kit (Qiagen). All RNA samples were treated with RNase-free DNase followed by RNA cleanup to eliminate DNA contamination. The RNA yield was determined on a NanoDrop-2000 spectrophotometer.

For RT-qPCR, 1.5 µg of each RNA and 50 pM of oligo(dT) primer were used for in vitro reverse transcription (RT) using High Capacity cDNA Reverse Transcription kit (ABI, Forster City, CA). SYBR-select master mix PCR kit was used to specifically detect target gene mRNA (ABI, Forster City, CA). Primer sequences for Mx2, IL1β, IFN-γ, and RPL18 were described previously (Zivcec et al., 2011). The primers for macrophage (MΦ) and dendritic cell (DC) marker CD68 (F, 5′-CCTGTCTCTCTCGTTTCCTTATG-3′, R, 5′-GTGGGAAGGACACGTTGTATT-3′), NK cell marker CD94 (F, 5′-CTCATCTCTAGTGTGCTTGGTG-3′, R, 5′-ATGGGACATGTTCTTTCAGGAG-3′), CD4 (F, 5′-CATCGTAACCCAGAACCAGAAA-3′, R, 5′-CCCTCGTATAGACTGTGGTAGAT-3′), and CD8α (F, 5′-GCAAGAAGAACGGTGACAAGTA-3′, R, 5′-CTGAAGTACACCACCGAGTTTC-3′) were designed using the PrimerQuest tool and synthesized by Integrated DNA Technologies (Coralville, IA). The PCR was set up in a 20 µl volume containing 1x SYBR select master mix (ABI), 250 nM forward and reverse primers, and 3 µl of the diluted RT