

# **Early host cell interactions and antivirals against ocular adenoviruses.**

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## **Akademisk avhandling**

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**Abstract**

Viruses are common causative agents of ocular infection among humans. Epidemic keratoconjunctivitis (EKC) is a severe and contagious ocular disease with reported outbreaks worldwide. It is estimated that this disease affects 20-40 million individuals every year, which leads to huge socioeconomic costs for the affected countries. EKC is characterized by keratitis and conjunctivitis but is also associated with pain, edema, lacrimation, and decreased vision that can prolong for months after the infection and in rare cases years. This disease is caused by human adenoviruses (HAdVs), which belong to the family of *Adenoviridae*. Currently, there is no available treatment against EKC. EKC is mainly caused by HAdV-8, HAdV-19, HAdV-37, HAdV-53, HAdV-54, and HAdV-56, which belong to species D HAdVs. HAdV-8, HAdV-19 and HAdV-37 have previously been shown to use sialic acid (SA)-containing glycans as cellular receptors to bind to and infect human corneal epithelial (HCE) cells. To characterize the receptor in more detail, we performed a glycan array, which included SA-containing glycans. A branched hexasaccharide terminating with SA in each arm was identified as a candidate receptor. This glycan corresponds to the glycan motif found on a ganglioside, GD1a. By performing a series of biological and biochemical experiments we confirmed the function of the GD1a glycan as a cellular receptor for EKC-causing HAdVs. However, the glycan used as a receptor was linked to plasma membrane protein(s) through *O*-glycosidic bonds, rather than to a lipid (as in the ganglioside). X-ray crystallography analysis showed that the two terminal SA:s interacted with two of the three previously identified SA-binding sites on the knob domain of the HAdV-37 capsid protein known as the fiber.

Based on the structural features of the GD1a:HAdV-37 knob interaction, we assumed that a three-armed molecule with each arm terminating with SA would be an efficient inhibitor. Such molecules were designed, synthesized and found to efficiently prevent HAdV-37 binding to and infection of corneal cells. These results indicate that trisialic acids-containing compounds may be used for treatment of EKC.

After binding to its primary receptor, most HAdVs have been shown to interact with  $\alpha V\beta 3$  and  $\alpha V\beta 5$  integrins to enter human cells. This interaction occurs through the RGD (arginine-alanine-aspartic acid) motif in the capsid protein known as the penton base. However, it was not clear if corneal epithelial cells express  $\alpha V\beta 3$  and  $\alpha V\beta 5$  integrins. Thus, to better understand additional early steps of infection by EKC-causing HAdVs, we performed binding and infection competition experiments using human corneal epithelial cells and siRNA, integrin specific antibodies, peptides and RGD-containing ligands indicating that  $\alpha 3$ ,  $\alpha V$ ,  $\beta 1$  affected HAdV-37 infection of but not binding to HCE cells. We could also see that HAdV-37 co-localize with  $\alpha 3$  and  $\alpha V$  at after entry into HCE cells. In situ histochemistry confirmed that the expression of  $\alpha 3$  and  $\alpha V$  in human corneal tissue. Overall, our results suggest that  $\alpha V$  and  $\alpha 3$  integrins are important for HAdV-37 infection of corneal cells.

Altogether, these results provide further insight into the biology of HAdVs and open up for development of novel antiviral drugs.

**Keywords**

Adenovirus, Virus host interactions, Antivirals, Sialic acid, Integrins, Epidemic keratoconjunctivitis

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