Title: ??

Originally discovered in Human cytomegalovirus (HCMV) Antigenic domain 6 (AD6) is a highly conserved structural motif of glycoprotein B (gB) found on all related herpesviruses and possibly much more broadly. In HCMV infection gB has been found to be essential for viral entry with a critical role in viral fusion, within this AD6 has been noted to undergo large structural changes during cell and viral membrane fusion. However, the triggers for this fusion are not properly understood with no cell or viral surface proteins yet been confirmed to catalyse fusion.

Previous published data showed that co-culturing HCMV AD6 with HCMV in human foreskin fibroblasts (HHFs) inhibited the amount of infection by competing for this unknown target but was not the case for an epithelia cell line Spontaneously arising retinal epithelial cells (ARPEs). In this study we expanded on these results to show that co-culturing Herpes simplex virus 1 (HSV1) with HSV1 AD6 inhibited infection of both ARPEs and HFFs.

Similarly, to as reported previously we found inhibition of HCMV infection by HCMV AD6 in epithelial cells but not fibroblasts. However, HSV1 AD6 was able to inhibit HCMV infection cross-reactively in HFFs only, suggesting it is capable of outcompeting HCMV AD6 to this unknown target.

As the different cell lines have different viral entry mechanisms inhibition of infection in one cell line and not the other would suggest a cellular target unique to HFFs that AD6 interacts with, or a viral protein specifically involved with the fusion process to HFFs specifically. Key considerations when developing novel anti-AD6 herpes virus vaccines.

**Ana’s rewrite**  
Suggested title: Using peptide neutralisation to unravel the function of a novel antigenic domain conserved across herpesviruses

Antigenic domain 6 (AD-6) of human cytomegalovirus (HCMV) glycoprotein B (gB) has a high level of structural and immunogenic conservation across different human herpesviruses (HHV). While the exact function of AD-6 remains unclear, we have previously shown that HCMV AD-6 peptide is able to neutralise HCMV infection. Here, we have further investigated the functional conservation of AD-6 across HHV by performing a set of equivalent experiments with HSV-1 and the corresponding AD-6 analogue peptide.

Interestingly, HSV-1 AD-6 analogue peptide was able to neutralise HSV-1 infection in both epithelial cells (ARPE-19) and primary fibroblasts (HFF). These data contrast with the previous HCMV AD-6 neutralisation where the peptide had no effect on infection in epithelial cells, indicating a potential difference in modes of cell entry and subsequent utilisation of gB within HHV. Intriguingly, HSV-1 AD-6 analogue was also able to show limited inhibition of HCMV infection, but not vice versa. Additionally, using alphafold-generated structures we were able to formulate a hypothesis of HCMV AD-6 interacting with another viral glycoprotein – gH – potentially outlining the mechanism of neutralisation.

Our findings suggest a conserved function of AD-6 in the context of HHV gB and highlight the importance of the domain for establishing HHV infection.

Word count: 199

**Latest version**

Suggested title: Using peptide neutralisation to unravel the function of a novel antigenic domain conserved across herpesviruses

Antigenic domain 6 (AD-6) of human cytomegalovirus (HCMV) glycoprotein B (gB) has a high level of structural and immunogenic conservation across different human herpesviruses (HHV). While the exact function of AD-6 remains unclear, we have previously shown that HCMV AD-6 peptide is able to inhibit HCMV infection. Here, we have further investigated the functional conservation of AD-6 across HHV by performing a set of equivalent experiments with HSV-1 and the corresponding AD-6 analogue peptide.

We were able to reproduce previous work showing that HCMV AD-6 was only able to inhibit HCMV infection in epithelial cells and primary fibroblasts. Furthermore, our preliminary data suggests, HSV-1 AD-6 analogue peptide was able to neutralise HSV-1 infection in both fibroblasts and epithelial cells. In fibroblasts both AD-6 analogues were able to cross-inhibit infection, from HSV-1 and HCMV, however, intriguingly, HSV-1 AD-6 analogue was also able to show limited inhibition of HCMV infection in epithelial cells, but not vice versa. Using alphafold-generated structures we were able to formulate a hypothesis that AD-6 has general conserved functions between HHVs but also virus specific functions and interactions that may explain key differences to the route of viral entry. Our findings are potentially outlining an important mechanism for viral entry and neutralisation to consider when developing AD-6 based HHV immunotherapies.

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Session Categories: Viruses: molecular machines to understand cellular processes

**Matt’s Version**

Suggested title: Investigating the role of a recently identified antigenic domain (AD-6) of glycoprotein B in herpes virus entry

Ongoing studies of the humoral immune response to a recombinant vaccine based on human cytomegalovirus (HCMV) glycoprotein B (gB) identified the novel antigenic domain 6 (AD-6) as an important correlate of protection. Recently, we have also shown that AD-6 is structurally and immunogenically conserved across gBs of the herpesvirus family. The precise role of AD-6 in gB function remains unclear, although we have previously shown that a recombinant HCMV AD-6 peptide is able to inhibit HCMV infection of fibroblasts suggesting a role for AD-6 in the entry process of HCMV and thus, potentially, other herpes viruses.

Here we confirm that HCMV AD-6 inhibits entry into fibroblasts but, interestingly, not into epithelial cells. In contrast, we observe that the HSV-1 AD-6 analogue peptide was able to neutralise HSV-1 infection in both fibroblasts and epithelial cells. Intriguingly, both AD-6 analogues were able to cross-inhibit infection of fibroblasts by HSV-1 and HCMV suggesting a shared mechanism. However, the HSV-1 AD-6 analogue also demonstrated partial inhibition of HCMV infection in epithelial cells, but not vice versa. We used alphafold-generated structures to predict differential binding of the AD-6 peptides to virion glycoproteins of HCMV and HSV and hypothesise that AD-6 has general conserved functions between HHVs but also herpes virus-specific functions and interactions that may explain important differences to their routes of viral entry. Our findings help to further understand the role of AD-6 as an important mechanism for viral entry and gB function to consider when developing AD-6 based HHV immunotherapies.

Hypothesis of HCMV vs HSV-1 entry in epithelial cells

HSV-1 requires gD to activate gH/gL which in turn activates gB for fusion, however gD must be released once gB is activated so AD6 must outcompete gD to bind gH/gL and therefore have a higher affinity. Therefore, the addition of HSV-1 AD6 peptide inhibits infection by blocking the binding to the target, HCMV cannot block the target as it has a much lower affinity.

HCMV does not require gD and so probably has a lower affinity so less AD6 peptide is bound gH/gL before entering the endosome when added and therefore infection is not inhibited. However, HSV-1 AD6 peptide has much higher affinity for the targets and therefore enough binds that in the endosome a concentration large enough to partially inhibit infection is present.

HSV-1 gD binds to the cell surface receptors including nectin and recruits gH/gL complex which in turn activates gB triggering virus fusion. in HSV-1 infection AD6 on gB is capable of binding gH/gL and gD at the site of nectin receptor binding. Therefore, the addition of AD6 peptide inhibits the attachment and recruitment and activation of gB and thus fusion cannot occur in in epithelial cells. GD is not present on HCMV but as AD6 is also potentially capable of binding gH/gL we believe that HSV-1 AD6 has a higher affinity than HCMV and so is present in the endosome at high enough concentrations.