Binding of decay accelerating factor by human echovirus 6 is a stable phenotype required for RD cell infection

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The prototype strain of human echovirus EV 6 (EV6) binds the complement regulator decay accelerating factor (DAF) with low affinity and, unlike other DAF-binding EVs, cannot haemagglutinate (HA) red blood cells. Binding to the surface of human rhabdomyosarcoma (RD) cells by EV6 is primarily mediated by the glycosaminoglycan heparan sulfate (HS). By passaging this virus in the presence of soluble heparin, a highly sulphated form of HS, we have generated HS binding variant viruses ($HS^{var}Vs$) that can mediate haemagglutination via DAF; HA of $HS^{var}Vs$ could be inhibited with very low concentrations of *Pischia*-expressed soluble DAF. Affinity chromatography using heparin agarose columns and binding-inhibition assays with soluble heparin demonstrated that these variants retained their HS-binding phenotype at levels indistinguishable from the wildtype virus. Sequence analysis of the region of viral RNA encoding the capsid proteins revealed single amino acid changes in the EF-loop of VP2 for each of the $HS^{var}Vs$, indicating that molecular determinants for DAF-binding are likely to reside in this region. An additional $HS^{var}V$, which demonstrated no detectable HS-binding phenotype, was found to have an additional charged amino acid change in the DE loop of VP1, which, when modelled on the 3D structure of the closely-related EV11, was found to form a ring of charged residues surrounding the pentameric apex. Interestingly, cell protection assays with an anti-DAF polyclonal antibody and/or soluble heparin revealed that, regardless of their HS binding phenotype, infection by prototype or variant viruses was completely blocked only in the presence of antibody, indicating a requirement of DAF for infection by these viruses. Furthermore, these viruses were shown to associate with DAF-containing lipid rafts at equivalent levels, supporting the conclusion that, while the HS-binding phenotype of these viruses may be variable, that of DAF-binding is stable.