Characterization of echoviruses that bind decay accelerating factor (CD55): evidence that some haemagglutinating strains use more than one cellular receptor


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Several echoviruses (EVs) have previously been shown to use decay accelerating factor (DAF) as a cellular receptor. Since DAF is expressed on erythrocytes, EVs that use this receptor cause haemagglutination. Here we show that all EVs that haemagglutinate do so via attachment to DAF and that this interaction can be inhibited by a monoclonal antibody (MAb) specific for DAF domain SCR3. Although the viruses haemagglutinate via DAF some can bind to rhabdomyosarcoma cells from which DAF has been removed and infect in the presence of a MAb against DAF. This suggests that some EVs have the capacity to interact with more than one cellular receptor.

Introduction

Echoviruses (enteric cytopathic human orphan) are picornaviruses of the genus Enterovirus. They share a common morphology of a small icosahedral capsid made up of 60 copies each of 4 virus proteins (VP1–4) surrounding a single positive-sense RNA genome of approximately 7500 bases. The majority of the 30 echovirus (EV) serotypes were isolated in the 1950s and 1960s from a range of clinically affected and asymptomatic individuals. The viruses are associated with diverse pathogenicity including diarrhoea, aseptic meningitis, rashes and respiratory disease (Melnick, 1990). This range of pathogenic manifestations of the EV group reflects a range of tissue tropisms that seems to be mediated, at least in part, by utilization of a range of cellular receptors for virus attachment and entry.

Two cellular receptors for EVs have so far been identified. EVs 6, 7, 13, 21, 29 and 33 have been shown to bind to CD55, decay accelerating factor (DAF) (Ward et al., 1994; Bergelson et al., 1994), a 70 kDa glycosylphosphatidylinositol (GPI) anchored glycoprotein involved in the regulation of complement activation and in cell signalling (for a review see Lublin & Atkinson, 1989); all are haemagglutinating (HA) viruses. EV1 binds to the $\alpha_2$ subunit of the $\alpha_2\beta_1$ integrin VLA-2 (Bergelson et al., 1992, 1993). The remaining serotypes, a number of which are described as haemagglutinating, remain uncharacterized with respect to the identity of their cellular receptor.

In addition to mediating virus attachment, some virus receptors perform additional functions essential to the initiation of a productive infection. For example, following virus binding, the poliovirus receptor triggers conformational changes in the virus particle and mediates entry via clathrin coated pits (Everaert et al., 1989; Holland, 1962; Kaplan et al., 1990). Some viruses however, have a requirement for other cell surface molecules in addition to the primary receptor. Our previously published data suggest that for EV7 a secondary factor is required during uncoating and entry after the virus has bound to DAF (Powell et al., 1997). We have also observed that antibodies against $\beta_2$-microglobulin block productive infection of some but not all cell types by this and other EVs although attachment is unaffected (T. Ward and others, unpublished). Foot-and-mouth disease virus (FMDV) uses the integrin $\alpha_v\beta_3$ (vitronectin receptor) for attachment (Bernstein et al., 1995). However, efficient infection of cells in culture also requires the presence of the cell surface proteoglycan, heparan sulphate (Jackson et al., 1996). Evidence is also accumulating that certain picornaviruses can use more than one cellular receptor. Among the enteroviruses, coxsackievirus A21 (CAV21) can use both DAF and intercellular adhesion molecule I (ICAM-I) for cell infection, as shown by the fact that antibodies to both molecules are required to completely block infection (Shafren et al., 1997a). The situation with coxsackie B viruses (CBVs), which can all mediate infection via the coxsackie and adenovirus receptor (CAR), is more complicated.
CBV serotypes 1, 3 and 5, can be ‘DAF adapted’ by selecting variants which have a higher affinity for DAF (Bergelson et al., 1995; Shafren et al., 1995). However, transfection of non-permissive Chinese hamster ovary (CHO) cells by CAR alone renders these cells permissive to all CBV strains, including the DAF-adapted variants (Bergelson et al., 1997). In contrast, CHO transfection with DAF does not confer permissiveness to any CBV serotype regardless of prior adaptation (Bergelson et al., 1997). This is consistent with the conclusion that other factors may be required for coxsackievirus infection via attachment to DAF.

To clarify further which of the EVs can bind to, and utilize, DAF for infection, we have used two approaches. Monoclonal antibody (MAb) 854, which was used to clone DAF (Ward et al., 1994), has previously been shown to block infection by a number of HA EVs (Ward et al., 1994). Monolayers of RD cells in a 96-well plate were incubated with MAb 854 at a 1:1000 dilution for 1 h at 37°C. As above, 10^5 TCID₅₀ virus was added to the plates and infection allowed to proceed for 24 h prior to staining. The symbols used are described above. HA reactions were performed by incubation of 2-fold serial dilutions of tissue culture supernatant in 50 µl with 50 µl of a 0.5% suspension of erythrocytes. In Table 1, ‘+’ indicates a standard HA reaction; ‘+’ indicates that the HA reaction was partial, transient, and only apparent at the highest concentrations of virus.

Methods

**Virus strains.** Echo- and coxsackie viruses were a kind gift from Brain Megson, Public Heath Laboratory Service, Colindale, London. The isolates had been obtained from the ATCC in 1988. The viruses were passaged in rhabdomyosarcoma (RD) cells.

**Expression of sDAF in *P. pastoris*.** The expression of sDAF in the *P. pastoris* yeast expression system has been described in detail previously (Powell et al., 1997). In brief, sDAF and sDAF deletion mutants were amplified by PCR from pCDM8 containing the complete DAF sequence. The sequence for a C-terminal (His)₉ tag was incorporated to permit affinity purification of the expressed protein on nickel resin. Amplified clones were digested with Xhol and EcoRI and ligated into pPIC9 (Invitrogen) followed by subcloning into pPIC9K using BamHI and EcoRI.

*P. pastoris* spheroplasts of the expression strain GS115 were transfected with linearized clone and selected for by growth on histidine free media. PIC9K contains the kanomycin resistance gene which permits the fusion of cell to the complete DAF sequence. The sequence for a C-terminal (His)₉ tag was incorporated to permit affinity purification of the expressed protein on nickel resin. Amplified clones were digested with Xhol and EcoRI and ligated into pPIC9 (Invitrogen) followed by subcloning into pPIC9K using BamHI and EcoRI.

**Neutralization of virus with sDAF.** sDAF was made up to volume at concentrations of 2 µM or 15 µM in PBS and incubated with 10^⁵ TCID₅₀ of virus. The virus–DAF mixture was incubated at 37°C for 1 h and applied to cells in a 96-well dish. The cells were incubated for 24 h and then examined for cytopathic effects by light microscopy after staining with crystal violet. In Table 1, ‘+’ indicates that the cell sheet was not infected as determined by the above observations; ‘+’ indicates that cell survival was clearly greater in the presence of DAF than in the control, but infection was not completely prevented by the presence of sDAF; ‘−’ indicates that cell survival was indistinguishable from that seen in mock treated control wells.

**Haemagglutination-inhibition assay.** MAb 854 was tested for the ability to inhibit haemagglutination by HA EVs. A 1:1000 dilution was serially diluted 2-fold in an HA plate in 50 µl of PBS; 50 µl of a 0.5% suspension of human O erythrocytes was then added. Binding of MAb 854 to the erythrocytes was allowed to proceed for 1 h at room temperature. Four HA units (1 HA unit is the amount of virus required to haemagglutinate 50 µl of a 0.5% suspension of human O erythrocytes in PBS) of EV3, 6, 7, 11, 12 and influenza A virus (X31) were then added to each well in 50 µl of PBS and the mixture agitated by gentle pipetting to resuspend the erythrocytes. HA reactions were complete at room temperature within 2 h and the plate photographed. Where the effects of sDAF were investigated an 8 µM solution was serially diluted 2-fold in an HA plate in 50 µl of PBS containing MAb 854 (1:4000, a concentration known to inhibit HA). Binding of sDAF and MAb 854 was allowed to proceed for 1 h followed by addition of erythrocytes as above.

**Inhibition of binding of 35S-labelled EV6 and EV7 by sDAF.** Approximately 10⁵ c.p.m. of radio-labelled EV6 and 7 was incubated with a range of concentrations of sDAF made up to volume in 50 µl of PBS. The virus–receptor mixture was incubated for 1 h on ice (sufficient for the binding to reach equilibrium; data not shown). This mixture was then incubated with one-eighth of a flask of RD cells (approx. 5 x 10⁶) for a further 1 h on ice. The cells were then harvested by centrifugation, unbound radioactivity removed, and the cells washed twice with PBS. The remaining radioactivity bound to cells was then quantified by scintillation counting. The percentage blocking observed was calculated relative to a mock treated control.

**Binding of 35S-labelled EV6 and EV7 to MAB 854- and phospholipase C-treated cells.** 35S-labelled EV6 or EV7 was bound to RD cells which had been pre-incubated with MAB 854 or phospholipase C (PIPLC). PIPLC treatment of cells was performed as previously described (Davitz et al., 1986). Briefly, 10⁵ RD cells were treated with 4 units of PIPLC (Sigma) made up in PBS–1 µM β-mercaptoethanol for 2 h at 37°C. RD cells were incubated on ice for 2 h with MAB 854 (1:1000 dilution of ascitic fluid). Binding of 35S-labelled EV6 and 7 was allowed to occur as described above; the bound radioactivity was then quantified, and expressed as a percentage of that bound to untreated cells.

**Binding of EV6, 6’ and 7 to WOP cells and WOP cells transfected with DAF.** Construction of the plasmid pCDM8 DAF has been described previously (Ward et al., 1994). Transfection of WOP cells
(a murine fibroblast line stably transfected with polyomavirus large T antigen that does not normally express DAF) with pCDM8 DAF was achieved by electroporation as previously described. In brief, 10^6 WOP cells were electroporated at 250 V and 250 µF with 50 µg/ml plasmid in 500 µl of HEPES-buffered saline. Transiently transfected cells were cultured for 48 h and binding assays performed with ^35S-labelled virus as described above.

**Infection of WOP cells and WOP cells transfected with DAF.** WOP cells were transfected as above and cultured as monolayers for 48 h. Transfected and mock transfected WOP cells were incubated with 5 RD p.f.u. per cell for 12 h. Infection was assayed by immuno-focal staining using a MAb directed against the capsid protein of enteroviruses (Dako). The transfection efficiency was likewise determined using MAb 854 and found to be approximately 5%.

**Results**

MAb 854 binds to domain 3 of DAF and has previously been shown to block infection by a number of EVs, including serotypes 7, 13, 21, 29 and 33 (Daniels et al., 1997; Ward et al., 1994). We have also demonstrated that expression in *P. pastoris* of a soluble derivative of DAF containing domains 2, 3 and 4 will block infection of susceptible cells by EV7 (Powell et al., 1997). Many enteroviruses including those previously reported to use DAF as a receptor are able to agglutinate human erythrocytes. DAF is known to be expressed on the surface of erythrocytes so it seemed highly likely that the HA phenotype of these EVs is a consequence of DAF binding. The possibility remained, however, that some EVs haemagglutinate by binding to other erythrocyte surface molecules. To clarify the situation, we investigated the use of DAF as a receptor by all strains of EV deposited in the ATCC and described as 'haemagglutinating' (see Table 1). Non-HA EV strains 1 and 9 were included in the experiments for comparison. In our assays some EVs, i.e. 6, 19, 24, 25 and 30, showed only weak or transient HA. EV6*, which is described as non-HA by ATCC, also showed weak HA in our assays.

**Table 1. Blocking of infection of RD cells by EVs and CBVs by the DAF-specific MAb 854 and sDAF at two concentrations**

<table>
<thead>
<tr>
<th>EV</th>
<th>MAb 854 (1:1000)</th>
<th>sDAF (2 µM)</th>
<th>sDAF (15 µM)</th>
<th>HA activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+/-</td>
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<tr>
<td>6*</td>
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<td>+</td>
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<td>+/+</td>
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<tr>
<td>7</td>
<td>+</td>
<td>+</td>
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<td>+</td>
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<td>33</td>
<td>+</td>
<td>+</td>
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<tr>
<td>CBV1</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>CBV3</td>
<td>-</td>
<td>+/-</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

**Non-HA EVs (ATCC)**

<table>
<thead>
<tr>
<th>EV</th>
<th>MAb 854 (1:1000)</th>
<th>sDAF (2 µM)</th>
<th>sDAF (15 µM)</th>
<th>HA activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6*</td>
<td>+/-</td>
<td>+</td>
<td>+</td>
<td>+/+</td>
</tr>
<tr>
<td>9</td>
<td>-</td>
<td>-</td>
<td>-</td>
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</table>

**MAb 854 and sDAF inhibit infection of RD cells by EVs**

The ability of MAb 854 and sDAF to inhibit infection of RD cells by a range of enteroviruses was assessed (Table 1). Only five of the tested serotypes (7, 13, 21, 29, and 33) were completely blocked by MAb 854. The same strains were also blocked by sDAF at a concentration of 2 µM. All the remaining HA serotypes (3, 6, 6*, 6†, 11, 12, 19, 24, 25 and 30) were blocked only in part, or not at all, by MAb 854 at the dilution tested (approximately 5-fold greater than that required to inhibit infection by an equivalent amount of EV7). Of these strains, all were blocked from infecting RD cells by sDAF, although most required a concentration of 15 µM to demonstrate an effect. The non-HA isolates, EV1 and 9, were not blocked by MAb 854 or sDAF. These results suggest that although some HA EVs are not blocked by MAb 854, they nevertheless bind DAF and can be neutralized by sDAF at high concentrations.

**Haemagglutination-inhibition of EV3, 6*, 7, 11 and 12**

MAb 854 has been shown to be directed against SCR domain 3 of DAF (Daniels et al., 1997). The fact that infection by some EVs is not blocked by this MAb but can be inhibited by sDAF raised the possibility that these viruses interact with domains other than SCR3. Indeed, it has recently been reported that CAV21 binds to DAF via domain 1 (Shafren et al., 1997). Another possible explanation is that the viruses can bind to DAF but are also able to recognize another receptor on RD cells and therefore infection of these cells is not inhibited by MAb 854.

To assess whether these viruses may attach to DAF domains other than SCR3, haemagglutination-inhibition reactions were performed in the presence of MAb 854 and with MAb 854 which had been pre-treated with a soluble derivative of DAF containing only SCR domains 2 and 3 (sDAF²,³).
EV3  854  854+sDAF
EV6  854  854+sDAF
EV7  854  854+sDAF
EV11  854  854+sDAF
EV12  854  854+sDAF
X31  854  854+sDAF

Fig. 1. Haemagglutination-inhibition of EV3, 6', 7, 11 and 12 by MAb 854. The ability of the DAF-specific MAb 854 to inhibit HA was tested. Erythrocytes were pre-treated with MAb 854 at 2-fold serial dilutions starting at 1:1000 (upper row). Similar HA reactions were performed in the presence of sDAF2,3. MAb 854 (1:4000) was pre-treated with 2-fold serial dilutions of sDAF2,3 starting at 8 µM. This mixture was incubated with erythrocytes prior to HA (lower row). The influenza virus strain X31, which haemagglutinates via attachment to sialic acid, was included as a negative control. Haemagglutination by these EVs is inhibited by MAb 854, demonstrating the involvement of DAF.

Representative results are presented in Fig. 1. We have previously shown that 2–3 domain constructs of sDAF react with MAb 854, but do not form a functional receptor for EV7 (Clarkson et al., 1995; Powell et al., 1997; data not shown). EV3, 6’, 7, 11 and 12 were selected because they showed strong HA activity and represented viruses requiring high and low concentrations of sDAF to block infection of RD cells (infection of RD cells by all these viruses except EV7 was not blocked by MAb 854; Table 1). MAb 854 inhibited HA by all these viruses and this inhibition was abrogated by pre-incubation of MAb 854 with sDAF2,3. The control influenza virus (strain X31), which haemagglutinates via sialic acid, was unaffected by these treatments. These data suggest that, like EV7, haemagglutination by EVs 3, 6’, 11 and 12 is via attachment to DAF. This conclusion is supported by the observation that sDAF alone will inhibit HA (data not shown). Moreover, since the HA can be inhibited by MAb 854, the data strongly suggest that the DAF–virus interaction involves SCR domain 3.

It seemed likely, therefore, that infection of RD cells by these viruses, which unlike HA is not blocked by MAb 854, is via an alternative receptor present on RD cells but absent from erythrocytes. The apparent neutralization of infectivity of these viruses by high levels of sDAF would, in these circumstances, be by steric hindrance of the second receptor binding site by bound sDAF. To explore this possibility we assayed the binding of radio-labelled virus to RD cells.

**Binding of radio-labelled virus to RD cells**

We have previously shown that sDAF can neutralize EV7 infectivity. However, incubation of EV7 with sDAF does not lead to irreversible virus neutralization and A-particle formation (Powell et al., 1997). The blocking effect is due to competition between sDAF and cell surface DAF for virus binding. The higher levels of DAF required to block infection of some EVs suggested that either the viruses have a higher affinity for cellular DAF relative to sDAF as compared to EV7, that there were significant differences in particle to infectivity ratios, or that some of the viruses had the potential to use an alternative receptor(s) which might differ from DAF in terms of abundance on the cell surface and/or virus binding affinity. The ability of sDAF to block the binding of 35S-labelled purified 160S particles of EV6 and EV7 to RD cells was investigated (Fig. 2). As shown in Table 1, EV6 displayed weak HA activity. Nevertheless, infection of RD cells by this virus was completely blocked by pre-incubation with 15 µM sDAF. Radio-labelled EV6 and EV7 bound efficiently to RD cells and this binding could be inhibited by sDAF. The sDAF IC50 for EV6 binding was found to be 3- to 4-fold higher than that for EV7 binding, confirming the earlier observation that higher concentrations of sDAF are required to block EV6 infection of RD cells as compared to EV7 (Table 1). This result excludes the possibility that differences in particle to infectivity ratios account for the differences in sDAF concentrations required to block infection. The result does not rule out the possibility that there may be a second receptor for EV6, in addition to DAF, on RD cells.

**Binding of EVs to cells pre-treated with MAb 854 or PIPLC**

As discussed above, a possible explanation for the fact that EV6 infection of RD cells can be inhibited by sDAF but not by MAb 854 is that the virus is also able to bind to an alternative cellular receptor. To investigate this possibility we assayed the
binding of radio-labelled virus to RD cells which had been pre-treated to effectively remove DAF. Since DAF has a GPI anchor, it can be readily removed from the cell surface using PIPLC. We also pre-treated the cells with MAb 854 on ice to sequester the DAF. Fig. 3 shows that both of these pre-treatments abolished 95% of EV7 binding. In contrast, the binding of EV6 and the EV9 and poliovirus controls was unaffected, and EV6’ was reduced by only about 20%. These results strongly suggest that DAF is not the sole receptor that can be used by EV6 and 6’ and that the isolates used in these experiments (which have been propagated in RD cells) probably do not use DAF as their principal receptor. It can be further concluded that their principal receptor(s) is not GPI anchored.

### Binding of EVs 6, 6’ and 7 to WOP and WOP/DAF cell lines

EV6 and 6’ are closely related viruses, but behave differently in respect to their neutralization by sDAF (Table 1). The ability of these viruses, together with EV7, to use DAF as a receptor for virus attachment was further investigated in WOP cells, which do not normally express DAF. In previous experiments we have demonstrated that neither MAb 854 nor EV7 bind to native WOP cells (Ward et al., 1994). WOP cells were therefore transiently transfected with DAF and 48 h later the ability of the cells to bind radiolabelled EV6, 6’ and 7 was measured (Fig. 4). Both EV7 and EV6’ bound efficiently to DAF-transfected WOP cells, and this binding could be blocked by pre-treatment of the transfected cells with MAb 854. EV6, on the other hand, did not efficiently bind to transfected WOP cells suggesting that this virus has only a low affinity for DAF. Low but significant binding of both EV6 and EV6’ but not EV7, to untransfected WOP cells was also observed.

The infection of both native and DAF-transfected WOP cells by all three viruses was investigated using an immuno-focal assay. EV6 and 6’ infected native WOP cells inefficiently (approximately 0.1% of cells became infected using 5 RD cell p.f.u. per WOP cell) and EV7 did not infect the cells at all. By

### Table 2. Infection of WOP cells and WOP cells expressing DAF with EVs 6, 6’ and 7

<table>
<thead>
<tr>
<th></th>
<th>NV</th>
<th>EV6</th>
<th>EV6’</th>
<th>EV7</th>
</tr>
</thead>
<tbody>
<tr>
<td>WOP</td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0 h</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>18 h</td>
<td>0</td>
<td>0.1%</td>
<td>0.1%</td>
<td>0</td>
</tr>
<tr>
<td>WOP/DAF</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>0 h</td>
<td>0</td>
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<td>18 h</td>
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<td>5%</td>
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</table>
contrast, transfected WOP cells were efficiently infected by all three viruses (the proportion of infected cells was approximately equal to the proportion transfected (~5%) as detected using an antibody against DAF) (Table 2). This result shows that DAF alone confers permissiveness to mouse cells for all three viruses including EV6, which is a weakly haemagglutinating virus and showed poor virus binding in the direct binding assay.

Discussion

A primary determinant of the cell tropism of a virus is its interaction with a specific cell surface receptor. The identification of virus receptors is therefore of fundamental importance to our understanding of virus pathogenesis. Evidence is accumulating that many enteroviruses use DAF as their cellular receptor (Ward et al., 1994; Bergelson et al., 1994, 1995; Karnauchow et al., 1996; Shafren et al., 1995, 1997a). Since DAF is expressed on erythrocytes, it was likely that enteroviruses that utilize DAF are among those previously described as haemagglutinating. However, the possibility remained that some of these viruses haemagglutinate via interactions with erythrocyte receptors other than DAF. We investigated all the HA EVs, making use of an anti-DAF MAb (MAb 854), and a soluble derivative of DAF expressed in the yeast P. pastoris. Previous reports have identified a number of EVs that use DAF as a receptor, including EVs 7, 13, 21, 29 and 33 (Ward et al., 1994) and 6, 11, 12 and 21 (Bergelson et al., 1994). However, several HA strains were omitted from these studies. The results presented in this paper indicate that all EV serotypes that haemagglutinate erythrocytes do so by interacting with DAF. This is shown by the fact that HA can, in all cases, be inhibited by either MAb 854 or sDAF. Since MAb 854 is directed against SCR domain 3 of DAF it is likely that in all cases interaction with the viruses involves this region of the molecule.

Our data also suggest that although certain EVs haemagglutinate via DAF, they also have the potential to infect cells via an alternative receptor(s). Obviously, the ability to use more than one receptor may significantly extend the cell tropism of a virus. Three lines of evidence support the notion that some EVs, for example 6 and 6', have the ability to bind more than one receptor. First, these viruses clearly demonstrate an affinity for DAF: infection of RD cells is blocked by sDAF, and HA is inhibited by both the DAF-specific antibody MAb 854 and sDAF. Secondly, although haemagglutination by all HA EVs is inhibited by MAb 854, infection of susceptible RD cells by several HA serotypes is not (Table 1). Moreover, binding of radio-labelled virus to RD cells is not inhibited by MAb 854 or by PIPLC treatment, which effectively removes DAF from the cell surface. Finally, the significantly increased levels of sDAF needed to block infection for some serotypes implies that they have a higher affinity for an alternative, uncharacterized, receptor.

We believe the most likely interpretation of these data is that these viruses can indeed use alternative cellular receptors. Inhibition by high concentrations of sDAF is probably due to steric hindrance of the virus surface, thereby preventing attachment to the alternative receptor. For these strains it is possible that DAF is not the principal receptor. To support this interpretation we included CBVs 1 and 3, for which at least two receptors have been identified (Bergelson et al., 1997; Shafren et al., 1995), and which exhibit a similar pattern of neutralization by sDAF (Table 1). These CBVs are not blocked by MAb 854, and are known to be able to infect via CAR. However, high levels of sDAF are capable of preventing infection of RD cells, presumably by competing with both cell surface DAF and CAR for the binding of virus. Our data suggest that EVs 3, 6, 11 and 30 can bind DAF, but seem also to recognize an alternative cell receptor with higher affinity. Some isolates of EV19 and 25 also fall into this category. EVs 6', 6'', 12 and 24 also seem to recognize more than one receptor, but their affinity for DAF appears to be relatively high. Because the affinity of these viruses for DAF seems to be subject to drift and selection, it is possible that different isolates of these strains may produce slightly different results.

It has been reported previously that DAF is the receptor for EV6 (Bergelson et al., 1994). Our data demonstrate that this virus does not bind efficiently to DAF expressed in WOP cells, but can infect these cells via DAF (Fig. 4 and Table 2). However, as can be seen from the data presented in Fig. 3, the binding of this virus to RD cells is unaffected by PIPLC treatment, indicating that it uses an alternative receptor in these cells. We have not studied the infection of HeLa cells by our isolate. Possible differences between laboratories may result from different passage histories of the viruses. The virus used by Bergelson et al. (1994) had apparently been adapted to grow in HeLa cells, whereas our virus has been passaged solely in RD cells since it was obtained from ATCC. Modifications to receptor binding properties as a consequence of cell adaptations have been observed previously for enteroviruses (Cova & Aymard, 1980; Reagan et al., 1984). It is interesting to note that in the Bergelson et al. (1994) study, anti-DAF antibodies did not totally block infection by HeLa-adapted EV6 and 12, but did totally block EV7 and 21 infection. This observation is consistent with the conclusions presented here.

Several EV serotypes used in this study displayed only ‘weak’ HA, which was often transitory in that it was apparent at 30 min after mixing the virus and erythrocytes but had disappeared by 2 h. These serotypes are listed as haemagglutinating by ATCC. Variability in HA phenotype has been observed in clinical isolates of certain EVs (B. Megson, personal communication). These observations are consistent with the conclusion that these viruses can use more than one receptor and may adapt rapidly to use predominantly one or the other in different clinical conditions or in different laboratory cultures.

By contrast, EVs 7, 13, 21, 29 and 33 show strong HA and
are inhibited completely by MAb 854. This suggests that for these viruses DAF is the sole means of attachment and the only receptor by which infection can be initiated.

The ability to bind DAF is widespread amongst fairly divergent members of the enteroviruses, including representatives that exhibit different pathogenesis. This suggests there may be an evolutionary advantage to using this regulator of complement activity. The nature of this advantage is worthy of further investigation.

We would like to thank Brian Megson, Public Health Laboratory Service, Colindale, London, for supplying echovirus strains and for helpful discussions.

References


Received 3 February 1998; Accepted 10 March 1998