Cellular receptors for viruses: links to tropism and pathogenesis

Jürgen Schneider-Schaulies
Institut für Virologie und Immunbiologie, Universität Würzburg, Verbacher Str. 7, 97078 Würzburg, Germany

Introduction

The interaction of a virus with its cellular receptor initiates a chain of dynamic events that will enable entry of the virus into the cell. In addition to the fact that the virus–receptor interaction is a multistep process in itself, multiple attachment receptors may be used sequentially, or in a cell-type-specific manner, and co-receptors may also be involved (for a review, see Haywood, 1994). It is beyond the scope of this article to give a complete review of cellular receptors for viruses. The data presented have, therefore, been selected to focus attention on those virus–receptor interactions that provide links to virus tropism and pathogenesis.

For most viruses, the receptor distribution in an individual is wider than the observed tissue tropism. Also, many virus–receptor interactions determine the host range and therefore constitute an interspecies barrier. Although mutations can accumulate rapidly, especially in RNA viruses, tolerable changes in the viral envelope proteins are constrained by the need to interact with a certain receptor. However, eventually, such changes may drastically alter the tropism and virulence of a virus. Furthermore, even in non-infected cells, the virus–receptor interaction may induce signal transduction, resulting in cytokine secretion, apoptosis, stimulation of the immune response, or, conversely, immunosuppression.

Virus tropism and pathogenicity are multifactorial

The term ‘entry’ for the first step of a virus life-cycle describes the various ways a virus can get into a cell and can be taken to mean pH-independent fusion at the cell surface, pH-dependent fusion in acidic endosomes, uptake of non-enveloped virions by endocytosis or the conformational changes leading to the uncoating of the viral genome (Fig. 1). The general concept of a ‘spring-loaded’ metastable state and conformational changes induced by low pH or binding of a virus to its receptor has been developed using influenza virus haemagglutinin (HA) and seems to be valid for a variety of viruses (for reviews, see Skehel et al., 1994; Steinhauer et al., 1992; White et al., 1994). Thus, HA switches from a native non-fusogenic to a fusogenic conformation, which is induced by exposure to acidic pH in the endosome. This conformational change can also be achieved by exposing HA to heat or denaturing agents such as urea, suggesting that the native structure of the molecule is trapped in a metastable state and that the fusogenic conformation is released by destabilization of the native structure. Coupling the energetically expensive process of membrane fusion to an energetically favourable conformational change drives the reaction (Carr et al., 1997).

Similar fusion (F)-protein structures have been found in the influenza virus HA, simian parainfluenza virus 5 F, human immunodeficiency virus (HIV) gp41, mouse moloney leukaemia virus envelope transmembrane protein, and Ebola virus gp2 protein (Baker et al., 1999 and references therein). Although structurally different, the tick-borne encephalitis virus (TBEV) E protein is also functionally analogous (Rey et al., 1995; Stiasny et al., 1996).

Although the virus–receptor interaction is needed to initiate infection, pathogenesis is a multistep process and the development of disease is influenced by the intracellular milieu, induced cell functions such as the capacity of the host to develop a proper immune response, the velocity of virus replication, cytopathogenicity and the spread of infection within and between organs, which again may or may not depend on the presence of specific cellular receptors. Even when infection does not proceed, virus–receptor-mediated signal transduction may induce the secretion of cytokines, such as interferons, which can have a great impact on the development of a disease. For example, enveloped viruses such as HIV and herpes simplex virus (HSV) can induce interferon-α via the mannose receptor on dendritic cells or monocytes (Milone & Fitzgerald-Bocarsly, 1998). This interaction induces signals in cells important for the immune system, but it does not lead to the uptake of viruses.

The budding and the release of viruses can also have a decisive impact on pathogenesis by determining the parameters of virus spread. This is obvious for pantropic viruses such as influenza or Sendai. The uptake and release of these viruses in polarized epithelial cells of the respiratory tract is restricted to the apical side. This is an important factor in preventing systemic infection. In contrast, viruses that are released basolaterally may spread systemically (for a review, see Compans, 1995).
The primary infection usually involves a small initial dose of virus and leads to little impact for the host. Replication of virus within the initial site of infection will then lead to a substantial multiplication of virus particles and considerable damage in secondary target organs. For the spread of virus to different organs and cell types, the use of multiple receptors might be advantageous. Viruses may either have the intrinsic capacity to use more than one receptor, as found for complex viruses such as herpes viruses (Tufaro, 1997), or they may change their capacity to bind to receptors or co-receptors by mutation of their envelope proteins. Both mechanisms are observed during HIV infection.

**Receptor usage and the pathogenesis of HIV**

CD4 has been found to act as the primary receptor for HIV (Klatzmann et al., 1984). In recent years, rapid progress has been made in identifying a set of chemokine receptors, which belong to a family of G-protein-coupled seven-transmembrane proteins, that represent co-receptors for HIV infection. Although not all factors are known yet, it appears that macrophage-tropic, T-cell-tropic and dual-tropic HIV strains use certain sets of co-receptors: macrophage-tropic strains primarily use CCR5, and T-cell-tropic strains primarily use CXCR4. In an infected individual, the co-receptor-determined tropism of the virus may change from macrophages or dendritic cells in mucosal tissues to a tropism for T cells. This change is due to mutations emerging in the viral envelope genes during progression of the infection (for a review, see Berger et al., 1999).

The concept of consecutive conformational changes is valid for HIV entry. The most favoured current model proposes that the initial binding of gp120 to CD4 induces a conformational change in gp120 which enables it to interact with a co-receptor. This interaction is supposed to induce a further conformational change in the viral envelope which results in the activation of gp41 and the positioning of its fusion active peptide in close proximity to the membrane and finally in the insertion of the fusion peptide into the membrane of the target cell. The dramatic conformational changes of gp120 have been confirmed by X-ray crystallographic studies (for a review, see Berger et al., 1999). As with influenza virus HA, the native HIV envelope protein gp120/gp41 complex appears to be trapped in a metastable state until the receptor interaction induces the transition to an energetically more favourable state. This conclusion is also supported by the enhancement of the infection with low concentrations of soluble CD4 or neutralizing antibodies which can facilitate structural alterations (Allan et al., 1990; Sullivan et al., 1995), and the inhibition of infection or cell–cell fusion by short peptides interacting with transiently exposed structures of the envelope proteins (Eckert et al., 1999 and references therein).

A major contribution to HIV pathogenesis (Fauci, 1993,
The non-enveloped capsids of picornaviruses can interact with a variety of cellular proteins (Table 1; Bergelson et al., 1994; Kuhn, 1997; Shafren, 1998; Triantafillou et al., 1999; Ward et al., 1994). Following binding, picornaviruses usually undergo several conformational changes leading to virus entry and uncoating of the genome (Arita et al., 1998; Dove & Racaniello, 1997; Kaplan et al., 1990; Racaniello, 1996). The geometry of the picornavirus and adenovirus interaction with the target cell surface is quite different. Depressions on the icosahedral capsids of some picornaviruses, the so-called canyons, were found to interact with cellular receptors, whereas attachment of adenoviruses is mediated by fibres with globular knobs at their tips projecting from the virus capsids. In spite of these structural differences, some viruses of both families compete for the same receptor, CAR (coxsackie virus–adenovirus receptor; Bergelson et al., 1997; Roelvink et al., 1998). CAR is a 46 kDa transmembrane protein belonging to the immunoglobulin superfamily and containing two extracellular domains. When transfected into hamster cells, this molecule mediates both attachment and entry of coxsackie virus B3 and B4 and adenoviruses 2 and 5 (Bergelson et al., 1997). The binding sites on the adenoviral fibre knobs for CAR have been identified recently, and might now be modelled to develop adenovirus vectors with altered tropism for gene therapy or as antiviral drugs (Bewley et al., 1999; Roelvink et al., 1999).

The attachment and uptake of serogroup C adenoviruses depends on two separate but co-operative events: the interaction of the fibre with an attachment receptor (CAR), and the interaction of the penton base with an internalization receptor, $\alpha_{v}\beta_{3}$ and $\alpha_{v}\beta_{5}$ integrins (Bergelson et al., 1997; Roelvink et al., 1998; Wickham et al., 1993). The penton base of adenovirus 2 may also interact with $\beta_{3}$-integrins as attachment receptors on the surface of haematopoietic cells (Huang et al., 1996a). Thus, CAR or $\beta_{3}$-integrins promote attachment of adenoviruses to cells, and $\alpha_{v}$-integrins mediate their internalization via endosomes. Interestingly, some of the coxsackie virus B strains which use CD55 (decay-accelerating factor, DAF) as attachment receptor also require co-receptors of the integrin family for virus entry (Agrez et al., 1997; Shafren et al., 1995).

In order to investigate the internalization mechanism, the three-dimensional structure of soluble recombinant $\alpha_{v}\beta_{3}$ molecules bound to adenovirus has been determined. The results suggest a precise spatial arrangement of the five arginine, glycine, asparagine (RGD)-containing protrusions on the penton base promoting integrin clustering and signalling...
Table 1. Viruses and cellular receptors discussed in this review

<table>
<thead>
<tr>
<th>Virus family (group)</th>
<th>Receptors</th>
<th>Distribution of receptors</th>
<th>Tropism of the virus</th>
<th>Associated diseases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenoviridae (subgroup A, C, D, E, F) adenovirus</td>
<td>CAR (lg superfamily) α4β1- and α6β4-integrins αvβ5-integrin</td>
<td>widely, monocytes</td>
<td>epithelial cells, lymphoid cells</td>
<td>respiratory infection, lymphoid tissues</td>
</tr>
<tr>
<td>adeno virus C2</td>
<td></td>
<td></td>
<td>respiratory infection, lymphoid tissues</td>
<td></td>
</tr>
<tr>
<td>Aenoviridae</td>
<td></td>
<td></td>
<td>febrile disease, lymphocytic choriomeningitis, persistent infection (mouse) Lassa fever</td>
<td></td>
</tr>
<tr>
<td>Coronaviridae (genogroup 1) HCV-229E</td>
<td>NRPN</td>
<td>widely</td>
<td>respiratory tract</td>
<td>common cold</td>
</tr>
<tr>
<td>TGEV (pig)</td>
<td>mPN</td>
<td></td>
<td>encephalitis</td>
<td>respiratory, enteric, hepatitis, neurologic, enteric infection</td>
</tr>
<tr>
<td>FIPV (cat)</td>
<td>mPN</td>
<td></td>
<td>hepatocytes</td>
<td>respiratory, enteric, hepatitis, neurologic, enteric infection</td>
</tr>
<tr>
<td>CoV (dog)</td>
<td>cAPN</td>
<td></td>
<td>respiratory tract</td>
<td>common cold</td>
</tr>
<tr>
<td>HCoV-CyA13</td>
<td>sialic acid</td>
<td>widely</td>
<td>respiratory tract</td>
<td>common cold</td>
</tr>
<tr>
<td>MHV (mouse)</td>
<td>IgR</td>
<td>widely</td>
<td>hepatitis, brain</td>
<td>respiratory, enteric, hepatitis, neurologic, enteric infection</td>
</tr>
<tr>
<td>BCoV (cow)</td>
<td>9-O-acetyl-neuraminic acid</td>
<td>widely</td>
<td>respiratory tract</td>
<td>respiratory, hepatitis,</td>
</tr>
<tr>
<td>ISV (chicken)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Filoviridae Marburg virus</td>
<td>asialo-glycoprotein receptor</td>
<td></td>
<td>hepatocytes</td>
<td>neutrophils, mononuclear cells, endothelial cells, hematocytes</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>haemorrhagic fever</td>
</tr>
<tr>
<td>Flaviviridae dengue virus</td>
<td>heparan sulfate</td>
<td>widely</td>
<td>monocytes/macrophages</td>
<td>febrile illness, haemorrhagic fever, or shock syndrome</td>
</tr>
<tr>
<td></td>
<td>HE4</td>
<td>?</td>
<td>monocytes/macrophages</td>
<td></td>
</tr>
<tr>
<td>HCV</td>
<td>glycoseaminoglycan (CD81 ?)</td>
<td>widely</td>
<td>hepatocytes</td>
<td>hepatitis, hepatocellular carcinoma</td>
</tr>
<tr>
<td>Hepatoviridae HBV</td>
<td>(HBV-binding factor)</td>
<td>widely</td>
<td>hepatocytes, FS/MC, other tissues</td>
<td>hepatitis, hepatocellular carcinoma</td>
</tr>
<tr>
<td></td>
<td>DHBV (duck)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Herpesviridae HSV-1, HSV-2</td>
<td>mannose-6-phosphate R heparan sulfate</td>
<td>macrophages, DC</td>
<td>epithelial cells, neurons</td>
<td>skin lesions, latent neural infection, encephalitis (rare)</td>
</tr>
<tr>
<td></td>
<td>HveA (TNFRSF14)</td>
<td>widely</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>HvecV2D (PVR family)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>EBV</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CD21 (CCP family)</td>
<td>B cells</td>
<td>B cells</td>
<td>infectious mononucleosis, Burkitt’s lymphoma, epithelial carcinoma</td>
</tr>
<tr>
<td></td>
<td>second unknown receptor</td>
<td>?</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Orthonervoviridae influenza A virus</td>
<td>sialic acid</td>
<td>widely</td>
<td>respiratory tract</td>
<td>influenza</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Paramyxoviridae measles virus</td>
<td>CD46 (CCP family)</td>
<td>widely</td>
<td>respiratory tract, mononuclear cells, endothelial and brain cells</td>
<td>acute measles, acute encephalitis, and SSPE (rare)</td>
</tr>
<tr>
<td></td>
<td>second unknown receptor</td>
<td>?</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CDV (dog)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>NDV (chicken) Sendai V (mouse)</td>
<td>sialic acid</td>
<td>widely</td>
<td>epithelial cells</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Picornavirus/ coronavirus (CV) A9 A9</td>
<td>α4β1-integrin, β1-microglobulin ICAM-1 (CCP family)</td>
<td>widely</td>
<td>gastrointestinal tract, other tissues</td>
<td>enteric infections, febrile illness, meningitis</td>
</tr>
<tr>
<td></td>
<td>A9 (CV A9)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>A9 (CV B)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>echovirus 1, 8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>echovirus 5, 11, 12, 13, 20, 21, 29, 30</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>HAV</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>HAVcO-1 (lg superfamily, mucin-like)</td>
<td>widely</td>
<td>gastrointestinal tract, liver</td>
<td>hepatitis</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>rhinovirus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ICAM-1, 2 (lg superfamily)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>poliovirus</td>
<td>PVR (lg superfamily)</td>
<td>widely</td>
<td>epithelial cells</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Retroviridae HIV</td>
<td>CD4 (lg superfamily) and chemokine receptors (TM7)</td>
<td>macrophages, T cells, dendritic, cells, microglia, brain cells</td>
<td>CD4+ positive cells, (m1-Tropic strains), few brain cells</td>
<td>lymphadenopathy, AIDS, encephalopathy</td>
</tr>
<tr>
<td></td>
<td>Ga/C (glycolipid)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FIV (cat)</td>
<td>CXCR4 (TM7 family)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Unless indicated in brackets, the viruses infect humans. For explanations of abbreviations see text of the review.

 events required for virus internalization (Chiu et al., 1999). The interaction of integrins with RGD-containing peptides can induce caspases and apoptosis (Ruoslahti & Reed, 1999 and references therein). It is not known whether viruses also induce such signals via interaction with integrins.

Adenoviruses have the ability to infect a wide range of tissues and have been identified as the cause of diseases such as respiratory infections, epidemic keratoconjunctivitis, pneumonia, cryptic enteric infection and gastroenteritis (Wadell, 1990). As suggested earlier, the different tropisms of the
Poliovirus: species tropism, but not tissue tropism, is determined by receptors

All three serotypes of poliovirus replicate initially in cells of the oropharyngeal and enteric tract. In the minority of cases (1–5%), virus then invades the CNS, destroys motor neurons and induces myelitis and paralysis. What role do cellular receptors have in the pathogenesis of the disease? The human poliovirus receptor (hPVR, CD155) belongs to the Ig superfamily. It contains three Ig-like extracellular domains and is expressed in four splice variants designated hPVR-α, β, γ, -δ (Bernhardt et al., 1994; Koike et al., 1990; Mendelsohn et al., 1989). Domain 1 of hPVR is sufficient to bind the virus (for reviews, see Nomoto et al., 1994; Racaniello, 1996; Wimmer et al., 1994). The viral receptor-binding sites are amino acids located on the floor and at the rim of the canyon-like depressions in the capsid (Colston & Racaniello, 1994; Liao & Racaniello, 1997). It has been suggested that antibodies may not be able to recognize epitopes in this narrow cleft of the canyon and therefore the development of neutralizing antibodies is hampered (Chapman & Rosmann, 1993). Thus, the topography of the receptor-binding site may directly contribute to the pathogenicity.

In man, PVR proteins are expressed in many cells and tissues, including the small intestine, lung, liver, heart, neurons of the spinal cord and the motor end-plate of skeletal muscles (Freistadt, 1994; Leon-Monzon et al., 1995; Nomoto et al., 1994). Since liver, lung and heart are not normally considered to be replication sites of poliovirus, this observation indicates that the susceptibility of tissues to poliovirus is not determined merely by the distribution of PVR. The susceptibility of PVR-positive mononuclear cells in the blood may play a role in the eventual CNS invasion (Freistadt et al., 1993). However, it remains to be investigated which of the PVR isoforms are expressed by which cell type and how the expression is correlated with infection during poliomyelitis. A mouse homologue of hPVR does not bind poliovirus, including those viruses adapted to mice. Thus, hPVR determines the species tropism of poliovirus (for a review, see Nomoto et al., 1994).

In hPVR-transgenic mice, poliovirus induces neurological defects similar to those observed in man. This is a perfect animal model to test the neurovirulence of various poliovirus strains (Deatly et al., 1999; Koike et al., 1991; Ren et al., 1990). Neurons, and not glial cells, were found to be infected and paralysis is caused by direct destruction of motor neurons. Interestingly, in both man and transgenic mice, the neurotropism of poliovirus cannot be explained solely by the tissue distribution of the receptor. Molecular determinants of neurovirulence and attenuation have been located in the 5’-proximal 1122 nucleotides of the viral genome (Macadam et al., 1993). Most mutations in this part of the genome do not alter the receptor usage, but they influence the interaction with intracellular factors. The internal ribosomal entry site within the 5’ non-coding region significantly contributes to host range specificity and neurovirulence (Gromeier et al., 1999; Shiroki et al., 1997).

Members of the poliovirus receptor family are also used by α-herpesviruses as cell entry mediators

Cell entry by α-herpesviruses, which have a double-stranded DNA genome of approximately 152000 bp and encode more than 100 proteins, is, in comparison to the smaller and simpler RNA viruses, a much more complex process. In most cases, five viral surface proteins (gB, gC, gD, gH and gL) interact with cellular receptors and mediate virus entry (for a review, see Spear, 1993). Several different cellular surface molecules have been identified as receptors which may act together, consecutively or independently, to effect the uptake of herpesviruses. These include heparan sulfate or chondroitin sulfate proteoglycans (Shieh et al., 1992; Shukla et al., 1999), mannone-6-phosphate receptors (Brunetti et al., 1995) and the herpesvirus entry mediators (Hve) A, B, C and D (Geraghty et al., 1998; Montgomery et al., 1996; Takahashi et al., 1999; Warner et al., 1998). HveA belongs to the tumour necrosis factor receptor family (TNFRSF14), whereas HveB, -C and -D belong to the PVR family. Heparan sulfate appears to be a necessary surface component for virus entry into primary neuronal cells (Immergluck et al., 1998). Is there a functional parallel between the receptor usage of poliovirus and α-herpesviruses resulting in the neurotropism of both viruses?

Heparan sulfate proteoglycans are used by several viruses as initial attachment receptors

The capacity of gD of α-herpesviruses to interact with a variety of receptors, including cell surface proteins of two different families and modified heparan sulfate, is astonishing. Why do these viruses use multiple receptors? Does it allow them to change their tropism in the course of an infection? Virus attachment to cells is normally mediated by gB and gC. However, gD is required for successful entry and is supposed to be involved in the activation of the fusogenic activity of the viral proteins gB and gH/gL. Wild-type strains of HSV-1 and -2 seem to be similar in the use of HveA or HveC for entry but they differ in the use of binding sites generated by sulfotransferases (3-OST-3) on heparan sulfate (Shukla et al., 1999). While the clinical manifestations of disease caused by the two serotypes are indistinguishable, there are differences in their epidemiology and pathogenesis (Corey & Spear, 1986a, b; Lafferty et al., 1987). HSV-1 is the usual cause of adult sporadic encephalitis, keratitis and oral mucocutaneous lesions,
whereas HSV-2 is more likely to cause genital lesions, meningitis and neonatal infections. This could be influenced by their differential receptor usage determined by the capacity of HSV-1 to interact with 3-OST-3-modified heparan sulfate (Shukla et al., 1999).

As well as α-herpesviruses, interaction with heparan sulfate has been demonstrated for HIV-1 (Mondor et al., 1998), human cytomegalovirus (Compton et al., 1993), foot and mouth disease virus (Jackson et al., 1996), dengue virus (Chen et al., 1997), Sindbis virus (Byrnes & Griffin, 1998), vaccinia virus (Chung et al., 1998) and adeno-associated virus type 2 (Summerford & Samulski, 1998). It seems possible that heparan sulfate could help these viruses to adhere to a cell before further higher affinity receptors induce adhesion strengthening and mediate entry (Haywood, 1994). This model is supported by results obtained using the flaviviruses, dengue virus and TBEV. Dengue virus binds first to heparan sulfate and then to a high affinity receptor, which induces endocytosis and subsequent cell membrane fusion (Chen et al., 1997; Hung et al., 1999; Putnak et al., 1997). Thus, heparan sulfate proteoglycans on the cell surface can be used as initial attachment receptors by several viruses.

### The tropism of the γ-herpesvirus

**Epstein–Barr virus is mediated by alternative usage of the complement receptor CD21 and an unknown receptor**

Epstein–Barr virus (EBV) glycoproteins gp350/220 mediate the attachment of virus to cellular receptors. These heavily glycosylated envelope proteins are the product of a single EBV gene, which is expressed as alternatively spliced RNAs (Beisel et al., 1985). CD21, the human receptor for the complement protein C3d, has been identified as a cellular receptor for EBV (Nemerow et al., 1986; Nemerow & Cooper, 1992; Tanner et al., 1987). CD21 is a single transmembrane protein of approximately 145 kDa containing 15 to 16 complement control protein (CCP) domains. The first two amino-terminal CCP domains of CD21 bear the virus-binding sites (Lowell et al., 1989). CD21 is expressed by B cells, follicular dendritic cells and a subset of thymocytes. Electron microscopic studies, as well as biochemical studies, have shown that EBV enters human peripheral blood B cells via large uncoated endocytic vesicles. In contrast to the entry pathway in primary B cells, EBV can directly fuse with the plasma membrane of target cells such as the B cell line Raji. Fusion is mediated by the EBV envelope protein gp85 (Nemerow & Cooper, 1992).

Interestingly, binding of EBV, or beads bearing the viral envelope proteins gp350/220, induce the capping and endocytosis of CD21 (Tanner et al., 1987; Tedder et al., 1986). Thus, as found for viruses such as HIV and measles virus (MV), infection of cells with EBV leads to the downregulation of its receptor, CD21, from the cell surface. The normal function of CD21 is not completely understood, but it certainly plays an important role in antigen-specific B cell activation and differentiation, processes which are disturbed by the interaction with EBV. After the binding of ligands, CD21 and the antigen receptor become associated in the B cell membrane. In addition, further cell surface molecules, such as CD19 and CD81, associate with CD21 and form a multimolecular complex, which is probably involved in signal transduction. Antigen localization and the generation of B memory cells are largely dependent on the presence of the complement protein C3, and soluble CD21 fragments can suppress the immune response in mice.

EBV, the causative agent of infectious mononucleosis, is closely associated with Burkitt’s lymphoma and several benign and malignant lymphoproliferative diseases occurring in immunodeficient individuals. The cell tropism of EBV in these diseases can be explained by the use of CD21 as receptor. However, the EBV genome is also found in epithelial cell tumours such as nasopharyngeal and gastric carcinoma which do not express CD21. Therefore, the presence of a receptor other than CD21 on epitheloid gastric cells has been suggested (Hsu et al., 1996; Kasai et al., 1994; Kim et al., 1998; Yoshiyama et al., 1997). A very efficient mode of infection with EBV, much more efficient (up to 800-fold) than infection with cell-free virus, is direct cell-to-cell spread (Imai et al., 1998).

### Measles virus: also more than one receptor

MV is a monocytic negative-strand RNA virus causing acute measles accompanied by a transient immunosuppression, acute post-infectious encephalomyelitis and CNS diseases based on MV persistence (for a review, see Griffin & Bellini, 1996). The envelope glycoproteins haemagglutinin (H), as a trimer, and the fusion protein (F), probably as a trimer, interact with receptors on the target cell surface (Langedijk et al., 1997 and references therein). A complement regulatory protein containing four CCP domains, CD46, was identified as a receptor for vaccine strains of MV (for reviews, see Dörrig et al., 1994; Gerlier et al., 1995). CD46 is expressed on almost all human cells except erythrocytes and cells in the CNS such as oligodendrocytes and a proportion of neurons and astrocytes (Johnstone et al., 1993; Ogata et al., 1997). Binding sites for MV H protein have been mapped to the first two extracellular CD46 domains (Buchholz et al., 1997; Casasnovas et al., 1999; Hsu et al., 1997, 1999; Manchester et al., 1997). MV infection of cells can result in the secretion of cytokines such as interferon-α/β, tumour necrosis factor-α, interleukin-1 and interleukin-6. Binding of MV to CD46 in the absence of infection can induce kinase-mediated signals in the contacted cells which contribute to the induction of cytokines (Ghali & Schneider-Schaulies, 1998; Wong et al., 1997). In the presence of interferon-γ, the synthesis of nitric oxide by macrophages is enhanced through the cytoplasmic domain of CD46 in response to MV infection (Hirano et al., 1999).
As in the case of EBV and HIV, MV can induce the down-regulation of its receptor from the cell surface after infection or after the extracellular contact of cells with MV H protein (Krantic et al., 1995; Naniche et al., 1993; Schneider-Schaulies et al., 1996). Since the natural function of CD46 is to act as a co-factor for the cleavage of the complement factors C3b and C4b, and to protect cells from lysis by autologous complement (for a review, see Liszewski et al., 1991), CD46-modulation by MV enhances the sensitivity of cultured cells to complement (Schnorr et al., 1995). In vivo, this may cause a rapid clearing of infected cells and thus contribute to the attenuation of such downregulating MV strains. Different MV isolates vary considerably in their capacity to interact with CD46. In contrast to vaccine strains, a number of recent wild-type isolates do not downregulate CD46 (Schneider-Schaulies et al., 1995a, b). This capacity is closely related to amino acids at positions 451 and 481 in the H protein (Bartz et al., 1996; Lecouturier et al., 1996).

Recently, B cell lines have been used to isolate a number of MV strains which obviously do not interact with CD46 (Bartz et al., 1998; Hsu et al., 1998; Tanaka et al., 1998). MV isolates that do not interact with CD46 have a preferred tropism for lymphoid cells. Studies using recombinant MVs (Radecke et al., 1995) with defined envelope proteins have confirmed these findings (Johnston et al., 1999). It remains to be seen whether MVs present during the acute disease in vivo interact with CD46, and what consequences the differential receptor usage might have for the pathogenicity of acute and persistent MV infections.

The most intriguing question at present is how cellular receptors are involved in MV-induced immunosuppression. During immunosuppression, the number of peripheral blood lymphocytes is reduced and activated T cells are depleted from the circulation (Nanan et al., 1999; for reviews, see Griffin, 1995; Schneider-Schaulies & ter Meulen, 1999). Ex vivo, the proliferative response of peripheral blood lymphocytes to antigens or mitogens is strongly inhibited. The MV-induced immunosuppression is a multi-factorial process which may be influenced by soluble factors such as IL-12 (Karp et al., 1996) and the direct contact of the viral H–F glycoprotein complex and the surface of lymphocytes (Schlender et al., 1996). The contact requirements for the proliferative inhibition of lymphocytes have been defined by using recombinant viral glycoproteins and recombinant viruses, and CD46 appears not to be required (Niewiesk et al., 1997a; Schlender et al., 1996). Recent data indicate that although a native H–F complex is obligatory, the process of membrane fusion is not required for inhibition of T cell proliferation (Weidmann et al., 2000). As in the case of HIV gp41, cellular receptors involved in the proliferative inhibition of lymphocytes have not yet been identified.

MV-induced CNS diseases and the mechanisms supporting virus persistence have been studied extensively (for a review, see Schneider-Schaulies et al., 1999). The influence of the virus attachment protein H on neurovirulence was investigated using antibody escape mutants in rats (Liebert et al., 1994) and in a mouse model using a recombinant MV in which the H gene of the Edmonston strain was replaced by the H gene of the rodent-adapted neuroviral neurovirulent MV strain CAM (Duprex et al., 1999a). After intracerebral injection into suckling mice only the recombinant virus bearing CAM-H, and not the virus with the Edmonston-H, induced a neurological disease. However, the neurovirulence of the recombinant virus was reduced compared to the wild-type CAM strain, indicating that other viral genes also contribute to CAM-induced pathogenicity.

Recently, CD46-transgenic mice have been used to define the role of the cellular receptor for the neurotropism and virulence of MV. In such animals the normally apathogenic Edmonston strain is able to cause widespread neuronal infection and death in neonates, and scattered infection of neurons of adult mice (Rall et al., 1997). These findings clearly demonstrate that expression of a suitable receptor on neurons can mediate neurovirulence. However, in the periphery of adult CD46-transgenic mice or rats, receptor expression did not lead to a significant increase of susceptibility for MV, suggesting an intracellular block of virus replication (Blixkenrøe-Moller et al., 1998; Horvat et al., 1996; Niewiesk et al., 1997b). In contrast, a different line of CD46-transgenic mice seems to be more generally susceptible to MV infection (Oldstone et al., 1999). Clearly, these differences in the susceptibility of different CD46 transgenic mice need to be further investigated. In interferon-α/β-receptor-deficient mice expressing a CD46 transgene, intracerebral inoculation of adult animals with low doses of MV caused encephalitis (Mrkic et al., 1998). These findings underline the importance of the interferon system and associated intracellular factors for the virulence of this virus.

Receptor interactions of lymphocytic choriomeningitis virus (LCMV) strains determine pathogenesis

Infection of mice with LCMV has been extensively studied as a model system for virus persistence, immune-mediated pathology and immunosuppression (Borrow et al., 1995). LCMV is the prototype arenavirus with a single-stranded, ambisense RNA genome of two segments, 7220 and 3376 nucleotides, containing four open reading frames. The receptor-interacting envelope glycoprotein G is cleaved to an external G1 and a transmembrane G2 glycoprotein. LCMV enters rodent target cells via receptor-mediated endocytosis and pH-dependent fusion (Borrow & Oldstone, 1994). A cellular receptor for LCMV has recently been identified (Borrow & Oldstone, 1992; Cao et al., 1998 and references therein). Interestingly, several strains of LCMV and other arenaviruses, including Lassa, Oliveros and Mobala fever virus, bound to the same protein, namely α-dystroglycan. Dystroglycan is encoded by a single gene and processed into α- and β-chains, which form a complex. The dystroglycan complex is
expressed in a wide variety of tissues and cells, and mediates interactions of cells with the extracellular matrix.

Persistent infection of C3H/St mice with certain clones of LCMV strain WE can cause growth hormone deficiency syndrome (GHDS). Reassortant studies indicate that a single amino acid exchange at position 153 in the viral envelope protein G1 is sufficient to allow infection of the growth hormone-producing cells and cause GHDS (Teng et al., 1996). It is not yet known whether this change in the virus tropism and pathogenicity is due to alterations in receptor usage of such clones. Another pair of LCMV strains, strain Armstrong and clone 13, have been found to cause differential immunosuppressive effects in mice (Borrow et al., 1995). Mice infected with LCMV Armstrong rapidly clear the infection, whereas mice infected with clone 13 develop a generalized immunosuppression associated with loss of interdigitating dendritic cells and failure to stimulate the proliferation of T cells. These two strains differ in only two amino acids, at positions 260 in the G1 protein and 1079 in the RNA polymerase. A difference in the tropism of the two strains has been found, which is probably caused by a stronger binding affinity of clone 13 to the LCMV receptor. This could enable clone 13 to infect cells expressing limited amounts of α-dystroglycan, or to infect cells more effectively than the Armstrong strain. These findings suggest an association between receptor usage and pathogenicity.

**Coronaviruses: receptors determine the host range**

Coronaviruses are a group of enveloped, positive-strand RNA viruses. The closely related human coronavirus (HCV) 229E, porcine transmissible gastroenteritis virus (TGEV), feline infectious peritonitis virus (FIPV) and canine coronavirus (CCV) belong to serogroup 1 and are antigenically and genetically distinct from the so-called haemagglutinating coronaviruses, such as HCV-OC43, murine hepatitis virus (MHV) and bovine coronavirus (BCV), which belong to serogroup 2, and the avian infectious bronchitis virus (IBV), which comprises serogroup 3 (Table 1; for a review, see Siddell, 1992). Other members of the CEA family in addition to Bgp1α, namely Bgp1β (Dveksler et al., 1993a, b; Yokomori & Lai, 1992), Bgp2 (Nedellec et al., 1994) and brain CEA (Chen et al., 1995) may also serve as receptors, with different binding efficiencies (Zelus et al., 1998). Bgp1α is the major receptor for MHV in susceptible mice, whereas Bgp1β, which is used less efficiently as a receptor, is expressed homozgyously by resistant SIL mice (Williams et al., 1990).

A change of receptor usage may be associated with the transition of the host range of coronaviruses from one species to the other. Conversely, experimental interspecies transfer of MHV also changed the receptor usage (Hensley et al., 1998). The S protein of MHV has recently been exchanged by targeted RNA recombination for the S protein of FIPV (Kuo et al., 2000). The resulting chimera virus acquired the ability to infect feline cells and lost the ability to infect murine cells, supporting the view that the host range of coronavirus infections is mainly determined by receptor usage.

**Do the known receptors for hepatitis viruses explain their liver tropism?**

A cellular receptor for hepatitis A virus (HAV) has been identified by screening a cDNA expression library of African green monkey kidney cells with an infection-inhibiting antibody (Kaplan et al., 1996). The HAV cellular receptor 1 (HAVcr-1) is a class I integral mucin-like membrane glycoprotein of unknown function (Kaplan et al., 1996; Thompson...
As shown for other picornaviruses, low pH during the uptake of HAV by receptor-mediated endocytosis induces conformational changes in its capsid which may be the first step to uncoating the HAV genome (Bishop, 1999). huHAVcr-1 is expressed in all human organs, with higher levels in the kidney and testis (Feigelstock et al., 1998). Since extrahepatic sites of HAV replication were found in the gastrointestinal tract, liver, salivary glands, kidney, spleen and lymph nodes of experimentally infected primates, the liver-specific pathogenesis of HAV cannot easily be explained (Asher et al., 1995). The detection of HAV in various organs may correlate with the expression of HAVcr-1. However, it is likely that unknown coreceptors or intracellular factors are required to render cells fully susceptible to HAV infection.

Human hepatitis B virus (HBV), an enveloped hepadnavirus with a partially double-stranded DNA genome of approximately 3200 nucleotides in length, binds to a 50 kDa binding factor (HBV BF) present in serum and on the surface of cells (Budkowska et al., 1993). HBV BF is a neutral metalloprotease which shares substrate specificity with a family of membrane-type matrix metalloproteinases. Treatment of HBV with the metalloprotease results in cleavage of the N-terminal part of the pre-S2 envelope protein, and probably induces a conformational change in the pre-S1 domain that enables cell membrane attachment and virus entry into T lymphocytes (Budkowska et al., 1997). Since an inhibitor of the metalloprotease blocked both processes, the host-dependent proteolytic activation of the envelope proteins seems to be essential for HBV entry into cells. The tissue tropism of HBV may be determined by this process of envelope activation and not by cellular receptors, which might be expressed ubiquitously (Budkowska et al., 1997; Köck et al., 1996).

An interesting mechanism has been proposed for the specific uptake of duck hepatitis B virus (DHBV) into hepatocytes using carboxypeptidase D (gp180) as a cellular receptor (Breiner et al., 1998; Kuroki et al., 1995; Tong et al., 1999; Urban et al., 1998). This peptidase is a Golgi-resident protein which is found only to a limited extent at the external cell surface. It cycles to and from the plasma membrane and in doing so, it may function as a carrier leading to the endocytosis of DHBV (Breiner et al., 1998). In spite of the high affinity binding of the DHBV surface protein to gp180, expression of gp180 in heterologous cells did not render them permissive to infection, suggesting that a species-specific co-receptor or cofactor is required for virus entry (Breiner et al., 1998). The highly specific infection of the liver by DHBV does not correlate with the ubiquitous gp180 expression, suggesting other mechanisms for targeting the virus to the liver.

Suramin, a polyanionic compound similar to heparin, was found to block HCV binding to human hepatoma cells at a concentration similar to that reported to be effective against dengue virus, suggesting an interaction of HCV with glycosaminoglycans on the cell surface (Chen et al., 1997; Garson et al., 1999). In addition, it was recently found that the E2 glycoprotein of HCV binds specifically to CD81, a transmembrane protein of the tetraspanin family (Pileri et al., 1998). It is not yet clear whether CD81 acts as a cellular receptor for attachment and entry of HCV into target cells. CD81 is involved in a number of biological responses including cell adhesion, proliferation and differentiation of T and B lymphocytes (Levy et al., 1998). Interestingly, the HCV E2 protein, after binding to CD81, induced aggregation of lymphoid cells and inhibited the proliferation of a B cell line (Flint et al., 1999). This might have important consequences for the pathogenesis of HCV. Since glycosaminoglycans and CD81 are expressed by a wide variety of cells, additional specific factors must determine the liver tropism of HCV.

Taken together, there is no obvious common receptor-mediated pathway for hepatic viruses and none of the cellular receptors known so far can explain the specific liver tropism and pathogenesis caused by these viruses. Interestingly, a receptor for Marburg virus, the asialoglycoprotein, is expressed specifically on liver cells, although this virus has a quite different tropism (Becker et al., 1995). Marburg and Ebola viruses infect a wide spectrum of cells including neutrophils, mononuclear cells, endothelial cells, fibroblasts and hepatocytes, and cause fulminant haemorrhagic fevers. Thus, this liver-specific receptor is used by a virus with a much broader tropism. The viral glycoprotein gene encodes three forms of the G protein: two secreted soluble forms, sGP and GP1, and the membrane bound GP1/2, which are involved in the virus-specific pathogenesis (for a review, see Klenk et al., 1998). The asialoglycoprotein receptor on hepatocytes may serve as a liver-specific receptor for Marburg virus, thereby explaining its liver tropism. However additional receptor molecules on other target cells must be postulated. The asialoglycoprotein receptor might be an attractive tool to target recombinant viruses as chemotherapeutic agents to the liver (Bitzer et al., 1997).

**Cell-to-cell spread of viruses contributes to pathogenesis, but may not depend on cellular receptors**

It was observed in the late 1960s that the MV ribonucleoprotein complex spreads in the CNS in the virtual absence of the viral envelope proteins and infectious particles. Cell-to-cell spread of MV was demonstrated in vivo and in tissue culture. It most likely involves localized fusion events at cell contact points, but CD46 is not required (Allen et al., 1996; Duprex et al., 1999b; Firsching et al., 1999; Lawrence et al., 2000; McQuaid et al., 1998; Meissner & Koschel, 1995; Urbanska et al., 1997). The velocity of virus spread also depends on the cytoplasmic parts of the viral glycoproteins and considerably contributes to pathogenesis (Cathomen et al., 1998a, b). In the case of canine distemper virus (CDV), another morbillivirus, antibodies to the cellular transmembrane molecule CD9 inhibit the CDV-induced formation of syncytia (cell–cell fusion), but not binding and uptake of virus (virus–cell...
The cellular fusion-regulating proteins (FRP)-1 and -2 play a role in cell–cell fusion. These proteins were initially found with monoclonal antibodies that stimulate the Newcastle disease virus (NDV)-induced cell–cell fusion (Ito et al., 1987, 1992; Ohgimoto et al., 1995). Interestingly, antibodies to FRP-1 stimulate NDV-induced and inhibit parainfluenza virus-induced cell fusion (Okamoto et al., 1997). Recent data indicate that HIV-induced cell fusion is also regulated by FRP-1, integrins and the activation of tyrosine kinases (Ohta et al., 1994; Tabata et al., 1998). In addition, HIV DNA may also spread from cell to cell in a CD4-independent fashion via apoptotic bodies (Spetz et al., 1999). Also, the syncytium formation of human T cell leukaemia virus type 1 is regulated in a cell-type-specific manner by ICAM-1, ICAM-3 and VCAM-1 and can be inhibited by antibodies to integrin β4 or β1 (Daenke et al., 1999).

Conclusions

Infection of cells by cell-free virus and virus spread from cell to cell are different processes which may depend on the presence of different cellular surface molecules. In infected organs, the cell-to-cell spread contributes significantly to the pathogenesis of a viral disease. Underlying mechanisms are not well understood and require more research in the future. There are several viruses for which the host-specificity depends on their cellular receptors, whereas a specific organ or cell tropism appears often to be influenced by still unknown factors. Experiments are being undertaken to define the potential of certain viruses to change their host ranges. These are necessary steps in determining biosafety in xenotransplantation and gene therapy. Signal transduction as a consequence of a virus binding to its receptor is an important mechanism that influences virus cytopathogenicity and the immune response. A major impact on pathogenesis is due to the immunosuppressive capacity of many viruses which in part can also be exerted via cell surface receptor interactions. The increasing number of systems in which recombinant viruses can be applied will help to further investigate such virus–receptor interactions and to define the consequences of these interactions for tropism, virulence and pathogenesis.

I thank Sibylle Schneider-Schaulies and Volker ter Meulen for helpful discussions and carefully reading the manuscript. My research is supported by the Deutsche Forschungsgemeinschaft.

References


Daenke, S., McCracken, S. A. & Booth, S. (1999). Human T-cell leukaemia/lymphoma virus type 1 syncytium formation is regulated in a cell-specific manner by ICAM-1, ICAM-3 and VCAM-1 and can be inhibited by antibodies to integrin $\beta_2$ or $\beta_3$. *Journal of General Virology* 80, 1429–1436.


wild-type glycoproteins: consequences for viral spread and cell tropism. *Journal of Virology* 73, 6903–6915.


induced down-regulation of CD46 is associated with enhanced sensitivity to complement-mediated lysis of infected cells. European Journal of Immunology 25, 976–984.


heparan sulfate proteoglycan is a receptor for adeno-associated virus type 2 viruses. *Journal of Virology* 72, 1438–1445.


