Galactose is needed only for expression of co-receptors used by Theiler’s murine encephalomyelitis virus as the virus does not directly bind galactose or use the UDP-galactose transporter as a receptor

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Theiler’s murine encephalomyelitis virus (TMEV) infects most mammalian cells, but a TMEV receptor has not been identified. Studies have demonstrated that the UDP-galactose transporter (UGT) is critical for TMEV attachment and entry into mammalian cells (Hertzler et al., 2001). It was suggested that UGT might function as a TMEV receptor. We have demonstrated that polyclonal rabbit antibodies to human UGT that cross-react with hamster UGT do not block binding to or infection of mammalian cells by either high- or low-neurovirulence TMEV. In addition, incubation of virus with galactose, or blocking galactose on the cell surface with lectins, does not inhibit TMEV binding or infection. Thus, TMEV needs UGT for its transporter activity and galactose for assembly of its co-receptors (attachment factors) but does not bind directly to galactose. Excluding direct involvement of UGT and galactose in TMEV binding and entry provides further insight into how TMEV interacts with the host cell and should facilitate ongoing studies to identify a TMEV receptor.

Theiler’s murine encephalomyelitis virus (TMEV), an enteric pathogen of mice, belongs to genus Cardiovirus, family Picornaviridae. TMEV consists of two neurovirulence groups based on disease in mice following intracerebral inoculation. High-neurovirulence GDVII and FA strains produce fatal encephalitis, while the low-neurovirulence BeAn and DA strains produce a persistent central nervous system infection in mice, which provides an experimental analogue for multiple sclerosis in humans (Lipton, 1975; Lehrich et al., 1976).

Virus binding to the surface of permissive cells is a major determinant of virus host range and tissue tropism. In the case of non-enveloped viruses such as picornaviruses, binding involves the recognition of unique structural features on the virion coat by host cell-surface protein(s). A prominent virion depression or receptor site, termed the pit, is present on the capsid, with VP1 loops I and II blocking lateral extension of this depression (Grant et al., 1992; Luo et al., 1992). Observations have indicated that the cardiovirus pit is involved in receptor recognition (Kim et al., 1990) and mutations in selected BeAn virus pit residues support this observation (Hertzler et al., 2000). The pit in cardioviruses is thought to be analogous to the canyon that circulates around the fivefold axis of polioviruses (Hogle et al., 1985), rhinoviruses (Rossmann et al., 1985) and coxsackie B viruses (Muckelbauer et al., 1999). These and other members of the Picornaviridae family have been demonstrated to use protein entry receptors (Evans & Almond, 1998; Rieder & Wimmer, 2002).

The protein entry receptor for TMEV is not yet known. A 34 kDa unidentified protein was found by virus overlay protein binding assay to be bound by both low- and high-neurovirulence TMEV (Kilpatrick & Lipton, 1991). Libbey et al. (2001) have shown that the peripheral nerve protein, PO, could function as a TMEV receptor in some cells, but this observation requires further confirmation. It has been postulated that TMEV uses at least two molecules for attachment and entry (Jnaoui & Michiels, 1999). It is possible that both neurovirulence groups use a common protein entry receptor but different attachment factors, or maybe even different protein entry receptors. Recently, Hertzler et al. (2001) demonstrated that TMEV needs the UDP-galactose transporter (UGT) for binding and infection, by using a BHK-21 ’receptor negative’ cell line (R26), selected through resistance to BeAn virus infection. These results were supported using a Chinese hamster ovary (CHO) mutant cell line, Lec-8, which is deficient in the UGT.
UGT is a member of the trans-Golgi network nucleotide-sugar transporter proteins associated with the biosynthesis of complex carbohydrates. Although UGT is a trans-Golgi-associated protein, some cycling to the cell surface may occur (Ladinsky & Howell, 1993; Shukla et al., 1999). The requirement of UGT for TMEV binding and entry in BHK-21 and CHO cells originally suggested that the transporter itself might function as a TMEV receptor (Hertzler et al., 2001). To examine a role for UGT as a receptor, a rabbit anti-human UGT antibody was used to block virus binding and infection. However, we first needed to demonstrate that rabir anti-human UGT recognized hamster UGT on the surface of BHK-21 cells. Since Aoki et al. (1999) were only able to detect human UGT after its overexpression, pCMVSPORT hamster UGT (Hertzler et al., 2001) was transfected into BHK-21 cells, which were examined by flow cytometry. As shown in Fig. 1(A), the human UGT antibody detected hamster UGT on the cell surface. Next, BHK-21 cells (1 × 10⁶) were incubated with 0-1 and 1 µg of polyclonal rabbit antiserum prior to binding and infection with the high-neurovirulence GDVII and low-neurovirulence BeAn viruses. [³⁵S]Methionine-labelled virus (2 × 10⁶ particles per cell) was incubated with UGT antibody-bound cells for 30 min at 4 °C and the supernatant and cell-associated radioactivity was determined for triplicate samples in a Beckman LS5000TD scintillation counter and plotted as the percentage of cell-associated counts. Neither antibody concentration inhibited binding of either virus to BHK-21 cells (Fig. 1B). UGT antibody-treated BHK-21 cells were also infected with both viruses at an m.o.i. of 10 and cell viability measured by MTT assay (Denizot & Lang, 1986). The percentage of lysed cells, i.e. cytopathology, was plotted at 16 h post-infection (p.i.) with uninfected cells as controls. As shown in Fig. 1(C), neither BeAn nor GDVII virus infection was inhibited by UGT antibody. These results indicated that UGT itself is not a TMEV receptor.

Since UGT is required for TMEV binding and infection (Hertzler et al., 2001) but does not function as a receptor, TMEV may either directly bind galactose or, as in the case of rotaviruses (Jolly et al., 2000), recognize galactose as a component of the cellular receptor. BeAn and GDVII viruses (m.o.i. of 10) were incubated with 1, 50 and 100 mM concentrations of galactose for 1 h at 24 °C prior to adsorption to BHK-21 cells. As shown in Fig. 2, there was no effect of galactose on virus binding, as determined by flow cytometry, or infection, shown by MTT assay of cell viability. Infection of BHK-21 cells in the presence of 100 mM galactose in the media also failed to inhibit TMEV binding and infection (not shown). These results suggest that galactose is not directly recognized by TMEV in binding to the cell surface.

Direct galactose binding was also examined using lectins that are oligosaccharide linkage-specific to inhibit virus infection. Peanut agglutinin (PNA) and jacalin preferentially bind galactose having galactosyl (β-1,3) N-acetylgalactosamine

![Fig. 1. Demonstration of cross-reactivity of anti-human UGT antibody with hamster UGT by surface staining (A), and comparison of BeAn and GDVII virus binding (B) and infection (C) of UGT-antibody-treated BHK-21 cells. (A) BHK-21 cells were transiently transfected with pCMVSPORT hamster UGT, harvested 24 h later, incubated with 1 µg rabbit anti-human UGT and FITC-conjugated anti-rabbit Ig for 1 h at 4 °C each and analysed by flow cytometry. A representative analysis is shown. (B) Cells (1 × 10⁶) were incubated without or with 0-1 and 1 µg UGT polyclonal antibody or 1 µg of an isotype control for 30 min at 4 °C. [³⁵S]Methionine-labelled virus (2 × 10⁶ particles) was bound to cells for 1 h at 4 °C, detected with a scintillation counter and plotted as percentage bound, using untreated cells with and without virus as controls. (C) BHK-21 cells were infected with GDVII and BeAn viruses at an m.o.i. of 10 with or without pretreatment with polyclonal UGT antibodies. The extent of CPE was determined by MTT assay at 16 h p.i. and the percentage CPE calculated, using uninfected cells as a control. Error bars represent the mean ± SD of three independent experiments.]
linkages present in O-glycan cores, and Maackia amurensis lectin I (MAL I) and Erythrina crystagalli lectin (ECL) bind galactose having galactosyl (β-1,4) N-acetylglucosamine linkages in N-glycan cores. BHK-21 cells were incubated with 10, 100 and 1000 µg of these lectins ml⁻¹ for 15 min at 4 °C prior to adsorption of either BeAn or GDVII virus (m.o.i. of 10) and virus cytolysis was measured by MTT assay at 16 h p.i. The percentage cytopathic effect was plotted, with uninfected cells serving as controls. PNA and ECL had no effect on virus cytopathology by either virus (Fig. 3A, D), whereas MAL I inhibited BeAn virus infection by more than 50 % (Fig. 3C). The inhibition by MAL I can be attributed to the fact that BeAn virus uses sialic acid on N-linked glycoproteins for attachment and entry (Shah & Lipton, 2002), the preferential binding site on galactose for this lectin. As expected, ECL, which recognizes α2,6-linked sialic acid, failed to block BeAn infection. None of the lectins blocked GDVII virus infection except jacalin at 1000 µg ml⁻¹; however, the reason for this result is unclear (Fig. 3B).

If UGT is not the receptor for TMEV and if there is no direct interaction between TMEV and galactose, then the only other possibility is that the protein entry molecule is glycosylated. The molecules that are known to require galactose for assembly or biosynthesis are sialic acids, glycolipids, glycoproteins and proteoglycans. Recently, the low-neurovirulence BeAn strain has been shown to use sialic acid on N-linked oligosaccharides for binding and entry (Fotiadis et al., 1991; Shah & Lipton, 2002). This observation is further supported by the CHO cell mutant, Lec-2, which has no sialic acid transporter and is resistant to BeAn infection (Shah & Lipton, 2002). These cells do not express sialic acid on the surface, but have galactose expressed and exposed on the cell surface (Deutscher et al., 1984).

High-neurovirulence GDVII and FA strains use heparan sulfate as an attachment factor to mediate cell entry (Reddi & Lipton, 2002). Heparan sulfate is linked to the protein core by a tetra-saccharide linker containing two molecules of galactose (Perrimon & Bernfield, 2000). A CHO cell proteoglycan-deficient mutant, pgsD-677, has been found to be resistant to infection by GDVII (Reddi & Lipton, 2002). D-677 expresses the protein core of the proteoglycan with only a part of the tetra-saccharide linker needed to add glycosaminoglycans – the part that has only xylose followed by two galactose molecules (Lidholt et al., 1992). Despite expression of galactose on the surface of the protein core, the resistance of these cells to GDVII infection also indicates that GDVII does not bind directly to galactose, but rather needs another moiety. Taken together, these data not only confirm that TMEV does not bind directly to galactose or use it for entry, but also indicate that TMEV uses glycosylated molecules for entry.

It is possible that viruses of both neurovirulence groups use a common receptor with different attachment factors. Recent studies demonstrating the use of sialic acid and heparan sulfate (molecules that need galactose for assembly) as attachment factors by BeAn and GDVII viruses, respectively, highlight the importance of both molecules. The
present data eliminating the involvement of UGT or galactose in viral binding and entry provide further insight into the interaction of TMEV with host cells and should help facilitate ongoing studies to identify a TMEV receptor.

ACKNOWLEDGEMENTS

We thank Shannon Hertzler who initiated these experiments and Hiroaki Segawa at the Tokyo Metropolitan Institute of Medical Sciences in Japan for providing antibodies. This work was supported by NIH grants NS 21319 and NS 23349.

REFERENCES


