Peripheral nerve protein, P0, as a potential receptor for Theiler’s murine encephalomyelitis virus

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Theiler’s murine encephalomyelitis virus (TMEV) belongs the family Picornaviridae. TMEV not only replicates in the gastrointestinal tract but also spreads to the central nervous system (CNS) either by a hematogenous or a neural pathway during natural infection. The DA strain of TMEV infects neurons during the acute phase, and glial cells and macrophages during the chronic phase, leading to a demyelinating disease similar to multiple sclerosis. Different virus-host receptor interactions in the peripheral and the neuronal cells could explain the pathways of viral spread from the peripheral to the CNS and neurons to glial cells. However, the receptor for TMEV remains unknown. P0 protein, a 28–31 kD glycoprotein, belongs to the immunoglobulin superfamily and constitutes 50% of the total myelin protein in the peripheral nerve. Other picornaviruses use members of the immunoglobulin superfamily as receptors. Thus we hypothesized P0 protein could act as a receptor for TMEV. In a virus overlay assay, radiolabeled TMEV bound to a 28–30 kD protein from the peripheral nerve of wild-type C57BL/6, but no binding was found in the peripheral nerve from P0-knockout mice. TMEV replicated fourfold higher in P0-transfected BW5147.G.1.4 cells than in mock-transfected cells. The increase in virus replication in the P0-transfected cell line was blocked by preincubation of the cells with anti-P0 antibody. A virus binding study showed that TMEV bound to P0-transfected cells but not to mock-transfected cells. The use of the P0 protein in Schwann cells as a receptor may be one mechanism by which TMEV spreads from the gastrointestinal tract to the CNS.

Keywords: Cardiovirus infections; myelin P0 protein; peripheral nerves; Picornaviridae infections; virus receptors

Introduction

Theiler’s murine encephalomyelitis viruses (TMEV) are naturally occurring enteric pathogens of mice (Theiler, 1937; Theiler and Gard, 1940). They are members of the family Picornaviridae and belong to the genus Cardiovirus. TMEV can be divided into two groups based on neurovirulence. The GDVII strain is highly neurovirulent while viruses in the TO group are less virulent and can cause a persistent central nervous system (CNS) infection with inflammation and demyelination. DA virus (within the TO group) induces demyelinating disease and is one of the viral models that mimics many human demyelinating diseases, such as multiple sclerosis (MS) (reviewed in Tsunoda and Fujinami, 1999; Tsunoda et al, 1998).

Similar to poliovirus in humans, in nature, TMEV is spread from mouse to mouse by the oral–fecal route (Theiler, 1937; Theiler and Gard, 1940). In order to cause CNS infection and disease by this route, the virus must get from the alimentary tract and spread into the CNS. Several hypotheses have been proposed to explain dissemination into the CNS. They involve either hematogenous spread or intra-axonal transport (Martinat et al, 1999; Ren and Racaniello, 1992; Racaniello and Ren, 1994; Tyler et al, 1986) or...
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a combination of both for the virus to get to the CNS. P0 protein is a 28–31 kD glycoprotein, which constitutes approximately 50% of the total myelin proteins in the peripheral nervous system. Expression of P0 is limited to myelinating Schwann cells (Brown and Lemke, 1997; Uyemura et al, 1992). P0 is a single-pass integral membrane protein. The extracellular domain is hydrophobic and contains an immunoglobulin (Ig)-like motif, which defines P0 as a member of the Ig gene superfamily (Yoshihara et al, 1991). The extracellular domain is thought to function in myelin compaction at the intraperiod line through homophilic interactions. The intracellular domain is basic and has been suggested to function in the compaction of myelin at the major dense line (D’Urso et al, 1990; Filbin and Tennekoon, 1992). A second function attributed to P0 is in neurite outgrowth in regeneration and repair (reviewed in Spiryda, 1998).

It is known that various other picornaviruses use members of the Ig gene superfamily as receptors. These include poliovirus and rhinovirus. Poliovirus recognizes the poliovirus receptor (PVR/CD155), an integral membrane glycoprotein, which is a member of the Ig gene superfamily (Bernhardt et al, 1994; Mendelsohn et al, 1989; Racanelli, 1996). The receptor for the major human rhinovirus is an Ig gene superfamily molecule, vascular cell adhesion molecule 1 (VCAM-1, CD106) on vascular endothelial cells derived from the heart as a receptor for the D variant of eencephalomyocarditis (EMC-D) virus that belongs to the genus Cardiovirus.

To date, only one possible receptor for TMEV has been reported but not identified or characterized (Kilpatrick and Lipton, 1991). Lipton and colleagues found that the BeAn (TO group) and GDVII viruses bind to several proteins separated from BHK-21 cells including prominent bands at approximately 33–34 kD and 18 kD. Kilpatrick and Lipton (1991) reported BeAn and GDVII viruses binding to these proteins as well. We also observed this with DA virus and found that DA virus was also able to bind to a 20 kD and 26 kD band. In BSC-1 cells (lane 2), there was some binding to a faint band at about 16 kD. The hippocampus (lane 3) shows little or no attachment of virus to any band. However, this may reflect our inability to obtain enough pure hippocampal material or the potential receptors are in low abundance or denatured since DA virus can efficiently infect hippocampal neurons in vivo (Wada and Fujinami, 1993). In spinal cord (lane 4), two prominent bands were observed to bind virus. One band has a similar migration pattern to the 33–34 kD protein of BHK-21 cells and the other at 16 kD. In cerebellum (lane 5) and in the brain stem (lane 6) a 16 kD band was also seen. The 16 kD band may not be a true receptor, since BSC-1 cells also have a band in this area and DA virus is not usually found in the cerebellum. Alternatively, the BSC-1 band and that observed in other tissue types are different proteins, which happen to migrate in the same area of the gel. Total brain extract (lane 8) had a similar binding pattern as

Results

Virus overlay assay

Virus overlay assays were performed using TMEV permissive BHK-21 and nonpermissive BSC-1 cell lines and tissues from SJL/J mice—a strain known to be susceptible to TMEV infection. As can be seen in

![Figure 1](Image)

*Figure 1* Virus overlay assay of two cell lines and tissues from SJL/J mice. Lanes are as follows: 1, BHK-21; 2, BSC-1; 3, hippocampus; 4, spinal cord; 5, cerebellum; 6, brain stem; 7, peripheral nerve; 8, total brain; 9, kidney. BHK-21 and BSC-1 are known to be permissive and nonpermissive to TMEV infection, respectively. Radiolabeled DA virus was detected on the membrane to which proteins from cell lines and tissues were transferred. Note a prominent 28–30 kD band in peripheral nerve (lane 7).

![Figure 2](Image)

*Figure 2* Coomassie stained SDS-PAGE gel of two cell lines and tissues from SJL/J mice. Lanes are as follows: 1, kidney; 2, marker; 3, BHK-21; 4, BSC-1; 5, hippocampus; 6, spinal cord; 7, cerebellum; 8, brain stem; 9, peripheral nerve; 10, total brain. Note a prominent 28–30 kD band in peripheral nerve (lane 9).