Characterization of protein involvement in rabies virus binding to BHK-21 cells

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Summary. Prior studies established the specificity of rabies virus receptors on BHK-21 cells based on the saturability of the receptors and on competitive binding. In the present study, we used protease-treated cells to identify the involvement of protein in the specific binding of rabies virus to these cells. In addition, biochemical characterization of n-octylglucoside solubilized BHK-21 plasma membranes demonstrated the involvement of a protease sensitive, heat insensitive, integral membrane protein or protein complex in rabies virus binding to these cells. The membrane component that binds rabies virus is associated with a high molecular weight fraction of the n-octylglucoside-plasma membrane extract isolated by gel filtration. This high molecular weight fraction (~450 KDa) is enriched with a cell surface integral membrane component that comigrates with denatured bovine serum fibronectin (220 KDa). This cellular component did not bind polyclonal antisera to fibronectin in Western blot (native or denatured) or immunoprecipitation experiments. Direct and specific virus binding to high molecular weight plasma membrane protein(s) separated on Western blots further confirmed the role of a protein receptor in rabies virus binding to these cells.

Introduction

The initial step in virus infection is the attachment of virus to the host cell [1–3], mediated by the interaction of the viral attachment protein (VAP) with one or more molecular constituent(s) on the host cell surface. The VAP of enveloped viruses is usually the surface glycoprotein which projects through and is anchored in the viral membrane. The host cell surface molecule(s) may act either as an intermediate by attracting the virus to the cell surface prior to its true receptor interaction (secondary binding), or it may directly constitute the cellular receptor unit (CRU) that is required for virus entry into the host cell [1, 4–6]. The premise that CRUs may determine virus host range [7] and tropism in animals [3, 8] has stimulated numerous

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investigations of cellular receptor specificities. We have used cell culture systems to examine cell surface receptor specificity for rabies virus. Rabies virus is a neuro-pathogen in vivo that initially infects muscle cells at the site of inoculation and then spreads via peripheral nerve fibers to specific neuronal cell types in the spinal cord and brain [9–12]. This restricted tissue tropism argues for the presence of specific receptors on surfaces of infected cells in vivo. Studies of nerve-enervated mouse diaphragm tissue and cultured chick myotubes exposed to rabies virus suggested that the nicotinic acetylcholine receptor (AChR), or a closely associated molecule, may serve as a viral receptor in vivo [13, 14]. These studies showed that rabies virus colocalizes with AChRs at neuromuscular junctions in the diaphragm model and in high density clusters in chick myotubes. Pretreatment of myotubes with α-bungarotoxin or d-tubocurarine, two nicotinic cholinergic antagonists that bind AChRs, decreased the number of rabies virus-infected myotubes, suggesting that the AChR was used by rabies virus to infect these cells. In subsequent experiments synthetic peptides were used to show that the rabies VAP binds directly and specifically to the α-subunit of the nicotinic AChR [15–17]. In a separate study, an anti-idiotypic antibody (B9) that mimics an epitope on rabies virus glycoprotein that neutralized rabies virus infectivity competed with rabies virus binding to cell lines expressing AChR, but not to cell lines lacking AChR [18]. In addition, mice immunized with the B9 antibody were protected from lethal intramuscular rabies virus challenge. These data further support the suggestion that the AChR may be biologically important in rabies virus infection in vivo.

However, not all cell lines susceptible to rabies virus infection express AChRs [19–21]. In addition, some neuronal cells infected with rabies virus in vivo may not express the AChR [20, 22, 23]. This raises the question of whether rabies virus uses more than one type of cell surface receptor. Indeed, other viruses recognize different receptors on different cells [6]. In addition, different receptors might facilitate attachment and penetration in a sequential manner, one receptor binding the virus before the second receptor is able to bind, or in a cooperative manner, both receptors binding virus simultaneously [6, 24–28]. AChR may act as the first receptor which binds rabies virus but not facilitate virus penetration, leaving the possibility that virus binding to other, as yet unidentified, receptors is necessary to complete the process of rabies virus penetration. Thus, it remains important to identify other molecules to which rabies virus may bind in order to develop a complete understanding of how the virus infects cells.

Previous studies from several laboratories using various cell culture systems have implicated both lipids and gangliosides in rabies virus binding to cells in culture [29–32]. Since rabies virus and vesicular stomatitis virus (VSV) binding to baby hamster kidney (BHK-21) cells were blocked by phosphatidylserine and since both viruses competed with one another for their receptor(s) on BHK-21 and mouse neuroblastoma (NA), clone 1300, cells, we suggested that rabies virus and VSV may share a common receptor on these cells [30]. An n-octylglucoside (OG) extract of whole BHK-21 cells blocked specific attachment of rabies virus and the blocking activity was variably sensitive to phospholipases. While these studies indicated that phospholipids or gangliosides mediated rabies virus binding to these cells, it was not