Rabies Virus Interaction with Various Cell Lines Is Independent of the Acetylcholine Receptor

Brief Report

By

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With 1 Figure

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Summary

Rabies virus infects most cells in vitro. The presence of the nicotinic acetylcholine receptor on the plasma membrane of various cell lines is not an obligate factor for rabies virus susceptibility of those cells.

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The restricted tissue involvement of rabies virus, a central nervous system (CNS) pathogen, in the early stages of infection remains a fundamental question of its pathogenesis. Elegant studies of rabies virus in its local, ascending, central, and centrifugal aspects of infection have used immunofluorescence and electron microscopy techniques to establish that the virus gains entrance to the CNS via peripheral nerve pathways (9–12). Indeed, neuronal ligation and axonal transport inhibitors (1) have effectively abrogated fatal infection of experimental animals. Since it is known that the initiation of a virus infection is dependent upon the successful binding of one or more infectious virus particles to cellular receptor units (CRU) in the plasma membrane (4), it is of interest to determine whether the observed host range restriction of rabies virus is due to an absence of CRU on tissues other than neurons and also whether specific entities on peripheral nerves productively attach rabies virus. To this end, Lentz and coworkers (8) have recently suggested that the nicotinic acetylcholine receptor (AChR) complex may serve as a cellular receptor for rabies virus, perhaps allowing a primary round of replication in skeletal muscle (10) followed by an uptake into the peripheral nerves. Two lines of evidence support their experimental interpretation. First, input rabies virus, as detected by indirect immuno-
fluorescence, was located at neuromuscular junctions of mouse diaphragm tissue infected with virus outside the animal (8, 16). Second, treatment of primary cultures of chick myotubes with high concentrations of alpha-bungarotoxin (α-Btx) or d-tubocurarine (dTC), both of which specifically compete with acetylcholine for the 40,000 molecular weight subunit of the AChR (5), reduced the number of myotubes that were infectable by rabies virus. We have tested the generality of these preliminary observations by determining whether the presence of the AChR on the cell surface membrane is a prerequisite for infection by rabies virus.

The model system we chose for this study was Jaffe's line of rat skeletal muscle cells, designated L8 (provided by M. Schultz, Hahnemann University), which differentiate with increasing cell density from unicellular presumptive myoblasts which lack high-density AChR to fused, multinucleated myotubes which elaborate high-density AChR (14). Also a clonal derivative of L8 cells, L8Cl3U, was used as a cell line which has lost its differentiating potential, and thus fails to form myotubes or develop AChR. It was assumed that if the AChR was necessary for attachment and internalization of rabies virus, then the susceptibility of these cells should parallel the availability of AChR. We also tested whether cells known to be susceptible to rabies virus in vitro possessed AChR (or entities which bind similar ligands). Thus, BHK-21 clone 13 cells (15), CER cells (7), mouse neuroblastoma clone 1300 (NA) cells (2), and Singh's mosquito cell line, Aedes albopictus (SAA) clone C6/36 (provided by S. Buckley, Yale Arbovirus Research Unit), were examined for the presence of AChR and their ability to develop rabies virus-specific immunofluorescent antigen. Cells (BHK-21, CER, NA, SAA, and L8Cl3U) were seeded into replicate 35-mm tissue culture plates (2 × 10^5 cells per plate), some of which contained coverslips. When the cells reached 80 percent confluency, they were either infected with the challenge virus standard (CVS) strain of rabies virus (3) at an input multiplicity of 10 PFU/cell or assayed for the presence of AChR. L8 cells (3 × 10^5/plate) were seeded into plates and similarly assayed after 24 hours (presumptive myoblast stage) and 216 hours (myotube stage) of incubation.

AChR, detected to at least the fmol level, were assayed on cells by the binding of [125I]α-Btx (13) (Amersham, 450 Ci/mmol). The binding assays for neurotoxin were performed in duplicate in the tissue culture dish in which cells were grown. The growth medium was removed and cells were washed once with toxin binding medium (minimal essential medium [MEM] containing 0.5 percent bovine serum albumin [BSA] and 30 mM HEPES, pH 7.5) and then overlayed with toxin-binding medium, either with or without dTC (1 × 10^-4 M). Cells were incubated with the overlay at room temperature for 15 minutes prior to the addition of [125I]α-Btx (final concentration was 1 × 10^-8 M) in the respective toxin-binding medium. Cells were incubated with labeled toxin for 1 hour at room temperature before the