Journal of NeuroVirology (1995) 1, 326–327

Guest editorial

Pseudorabies virus as a neuroanatomical tracer

Neuroscientists became interested in pseudorabies virus (PRV) after it was proven to be a useful tool for transneuronal tract tracing. PRV has many advantageous characteristics over classical anatomical tracers, such as neurotropism, preferential retrograde directionality, self amplification that results in an equally strong signal in primary and transneuronally labeled cells, and transneuronal labeling of at least three neurons in a chain. Because PRV gives a stronger signal than a classical tracer, it has the potential of revealing neural systems more extensively than classical tracers. Finally, the fact that PRV infects rodents but not humans makes it a safe tool for tracing studies.

A number of questions remain unresolved concerning the use of this virus, the first being the effect of viral dose on the outcome of infection. Loewy has reported that an inoculum of more than 4000 pfu of PRV per peripheral injection site will increase the probability of 'non-specific' spread in the CNS, a non-synaptic transmission of the virus (Loewy, 1995). There are yet no available data to confirm this dose dependent differential viral spread. After a peripheral injection, PRV reaches the CNS through neural pathways that innervate only the inoculated area. This has been demonstrated in experiments where inoculation of the skin or muscles of a denervated rat hindlimb with 7×10^6 pfu does not lead to any viral disease or detectable virus immunoreactivity in the CNS (Jasmin, unpublished observation). If there were to be 'non-specific' spread of the virus, it could conceivably be due to the infection of additional neurons when large inocula of PRV are used. A larger number of virions could increase the chances of viral uptake by the axon terminal. Alternatively, a larger number of virions reaching the cell body from the terminals could lead to an increase in permissivity of replication.

From this it follows that the question of what determines viral uptake into a cell must be addressed. PRV entry is reasonably well understood in tissue culture, but the molecular basis for infection and spread in neural tissue is only now being analyzed. In order for PRV to bind efficiently to cells in culture, the PRV gC envelope glycoprotein must first interact with extracellular matrix proteins like heparan sulfate proteoglycans prior to interaction with a secondary receptor on the cell surface. Deletion or mutation of the gC PRV gene, however, does not block the entry of the virus in murine fibroblast (Karger *et al*, 1995). Thus, other unknown attachment strategies may be involved, that may or may not use either viral gC or heparan sulfate. In support, Card and his colleagues have shown that the Bartha strain of PRV, which has a variety of mutations including defects in the gC gene, will replicate in only a subset of retino-recipient neurons of the superior colliculi. It remains to be investigated whether the Bartha strain propagates via non-heparan sulfate pathways, especially since there are variations in heparan sulfate regional distribution in the CNS (Fuxe *et al*, 1994). To further complicate the issue, certain neural pathways are resistant to infection by PRV. This has been shown for the primary sensory neurons of the olfactory pathway and the trigeminal mesencephalic nucleus. On the other hand, some neurons, such as the sympathetic neurons, are exquisitely sensitive to infection and thus are a preferential route for spread of PRV infection. The basis for variable susceptibility to PRV infection in different neural pathways has not yet been described.

Another question is, can transmission of PRV between neurons occur non-synaptically? There is substantial evidence for synaptic transmission of PRV-Bartha. The studies of Strack and Loewy (1990) on the superior cervical ganglion efferents, and the EM study of Card and colleagues (1993) on transmission in the dorsal nucleus vagal and efferents, favor synaptic transmission only. In the second study, infected neurons were shown to be isolated from the surrounding neuropile by reactive astrocytes. Thus, virions budding out of the infected neurons at non-synaptic sites most likely infect neighboring glia, where an abortive viral cycle occurs, and further dissemination of the virus is prevented. It should be mentioned that the virions reaching the pre-synaptic membrane area are probably actively transported there. It remains unknown through which mechanism (transport system) virions might reach non-synaptic sites if they do. Many other questions on the factors which influence the spread of PRV remain to be answered. What are the factors that determine the fraction of PRV that is cleared from the injection site, thus reducing the number of particles available to nerve fibers? In which neurons and under which conditions will PRV become lytic or latent? PRV clearly causes neuronal death but when does it occur in relation to the bulk of synaptic transmission from the infected cell?

To address some of these questions, Card and colleagues in this issue examine the critical dose of viral inoculum necessary to trace the visual pathways for two strains of PRV, the virulent Becker strain and the more attenuated Bartha strain. They convincingly demonstrate the influence of two

parameters, inoculum and strain, on anatomical tracing studies. The results of this study imply that anatomical tracing with the Bartha strain should be interpreted as an underestimation of the neural pathways projecting to an injection site. This selectivity of the Bartha strain could be an advantage for gene transfer strategies targeting a specific population of neurons, but is a clear limitation for neural circuit analysis.

In a separate article, Standish and colleagues demonstrate labeling obtained with a lacZ recombinant PRV-Bartha (engineered by Miselis in Enquist's laboratory). Until now, most investigators have used immunocytochemistry with a primary antibody directed against viral proteins to detect PRV in tissue sections. Standish shows that detection with an antiserum to the reporter gene β-gal results in more extensive labeling of the dendritic arborization of PRV infected cells. The mechanism by which β-gal as an antigen better labels the distal dendrites than viral proteins remains to be elucidated. With this new technique for detecting PRV, one can design experiments in which it will be possible to combine transneuronal labeling of neurons with anterograde labeling of their dendritic afferents. Together, these two reports further define the potential of PRV as an anatomical tracer.

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