Rabies virus infection and transport in human sensory dorsal root ganglia neurons

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Cultured human sensory neurons are directly susceptible to CVS rabies virus infection and produce virus yields of $10^5$ p.f.u./ml; infection can persist for more than 20 days without any sign of c.p.e. The use of a compartmentalized two-chamber culture system, with access to either the cell soma or neuritic extensions, permitted the study of viral retrograde transport, which occurs at between 50 and 100 mm/day. Neurons of human origin were more susceptible to virus infection than rat neurons and the axonal transport of rabies virus was more efficient. Electron microscopy allowed virus transport and infection of human dorsal root ganglia neurons to be observed.

The interactions of rabies virus with host cells have been described in several reviews (Murphy, 1977; Tsiang, 1988). Neuronal susceptibility to rabies virus has been demonstrated by electron microscopic observations of cells from natural animal hosts and humans (Matsumoto, 1975; Charlton & Casey, 1979; Iwasaki et al., 1985); however, the susceptibility of primary neuronal cultures to rabies virus infection in vitro has been described only for rodents (Tsiang et al., 1983, 1986), as has the retrograde and anterograde axonal transport of virus in rat dorsal root ganglia (DRG) neurons (Lycke & Tsiang, 1987; Tsiang et al., 1989). The crucial role of axoplasmic transport of virus for the dissemination of rabies virus in rodent brains has also been demonstrated (Gillet et al., 1986; Ceccaldi et al., 1989, 1990; Coulon et al., 1989; Kucera et al., 1985). Therefore it was of interest to investigate the susceptibility of cultured human primary neurons to rabies virus infection and their capacity to support viral transport.

Fixed CVS rabies virus was prepared from the supernatant of infected baby hamster kidney cells as described elsewhere (Tsiang et al., 1986). Human embryo (10 to 12 weeks gestation) DRG were obtained either from the Göteborg hospital (Sweden) or the Kremlin-Bicêtre hospital (France). Dissociation of ganglia, preparation of cultures and neuronal identification were performed as described previously (Lycke & Tsiang, 1987; Lycke et al., 1988).

Replication of rabies virus in cultures of dissociated human sensory neurons from DRG was monitored by fluorescence studies (Fig. 1) and determination of the virus titre in the supernatant (Fig. 2). A peak virus titre was observed on day 2, with an average titre of $10^5$ p.f.u./ml; the virus titre decreased slightly in the following 2 days. In infected cultures maintained for a longer period of time, no particular morphological changes were observed over a 20 day observation period, but the virus titre gradually decreased from $10^5$ to $10^4$ p.f.u./ml.

In transport experiments, the cell soma of human DRG neurons (internal compartment) produced neurites which extended across a silicone barrier into the external compartment (Fig. 3). Their extension was directed by the presence of a typical growth cone visible at the end of each neurite. Infection of these neurites in the external compartment resulted in the production of about $10^3$ p.f.u. virus/0.2 ml from the internal, somatic compartment as early as 18 h post-infection (p.i.), a titre which steadily increased ($5 \times 10^4$ p.f.u./0.2 ml on day 4) (Fig. 4). Using similar conditions, the release of virus into the internal compartment of rat DRG cultures was detectable in similar amounts ($10^3$ p.f.u./0.2 ml) only on day 3 p.i. and did not increase significantly in the following days.

Electron microscopic observations showed that virions were present close to microtubular structures soon after infection (Fig. 5). Whether these virions were inside vesicles or free in the cytoplasm was not clear. Cytoplasmic viral inclusions appeared in the neuronal cell body 1 to 2 days p.i. and structures typical of virus budding from the plasma membrane were observed (unpublished data).

Two aspects of rabies virus infection of human DRG neurons must be pointed out. First, cultured human
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DRG neurons are highly susceptible to rabies virus infection and, second, virus transport is more efficient than that in rat DRG neurons. Persistence of infection in DRG neurons in the absence of c.p.e. seems to be a characteristic shared by several neurotropic viruses (Lycke & Tsiang, 1987; Lycke et al., 1988; Löve et al., 1987) and demonstrates the capacity of the sensory neurons to allow virus replication for extended periods of time.

Our previous experiments on the transport of rabies virus in the rat brain (Gillet et al., 1986), as well as reports by others (Kucera et al., 1985; Coulon et al., 1989), have demonstrated the predominant role of axonal transport in the delivery of rabies virus to its natural target cells. Inhibition of virus transport by colchicine shows that the integrity of microtubules is a prerequisite for rabies virus transport in neurons in vitro (Lycke & Tsiang, 1987) and in vivo (Ceccaldi et al., 1989, 1990).

Fig. 1. Fluorescence studies of rabies virus-infected human DRG neurons in culture. Infected neurons from the inner chamber of the compartmentalized cultures were stained with fluorescein isothiocyanate-labelled anti-rabies virus nucleoprotein IgG 3 days p.i. Viral inclusions are present in the cell soma as well as in the neuritic extensions. Bar marker represents 5 μm.

Fig. 2. Kinetics of virus replication. Cultured human neurons (1 week old) were infected with 10^6.5 p.f.u./ml CVS rabies virus and samples were taken at different times. Virus titres were determined by plaque assay.

Fig. 3. Neuritic extension in the external compartment of the two-chamber culture system. The neurites could be observed 10 days after seeding of dissociated cells into the inner compartment. In this compartment the neurites are slender and never associated with Schwann cells. Bar marker represents 2 μm.
These data strongly suggest that the role of extracellular virions in dissemination of the virus, as shown by electron microscopy (Charlton & Casey, 1979; Iwasaki *et al.*, 1985; Murphy *et al.*, 1973), must be minimal. Thus the role of neurons in virus transport is even more important than previously suspected. The budding of virus from the post-synaptic membranes of infected cells and simultaneous virus uptake by a presynaptic axon terminal is probably the mode of transport of virus from an infected neuron to an uninfected neuron. Indeed, evidence of such a phenomenon has been obtained from electron microscopy in *vivo* (Charlton & Casey, 1977). The difference between somatic and neuritic infection of rat and human DRG neurons indicates that the intracellular traffic of rabies virus is not random and that different pathways are involved. A similar process was observed during the anterograde transport of rabies virus, which occurs at two different transport velocities (Tsiang *et al.*, 1989). These data may be related to the differential targeting of the vesicular stomatitis virus temperature-sensitive mutant and the wild-type avian influenza fowl plague virus glycoproteins to either the dendritic domain or the axonal membranes of polarized hippocampal neurons (Dotti & Simons, 1990).

In conclusion, the *in vitro* experimental model we used has demonstrated for the first time the high susceptibility to rabies virus infection of primary human neurons, which are one of the natural target cells in human infection.

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### References


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