Guest Editorial

Rabies pathogenesis

Alan C Jackson

Departments of Medicine and Microbiology and Immunology, Queen's University, Kingston, Ontario, Canada

Rabies is as old as antiquity, but the disease remains an important public health problem today. Our understanding of the pathogenesis of this disease comes mostly from studies using experimental animals, particularly rodents, and fixed strains of rabies virus (Jackson, 2002). As such these studies do not represent natural conditions where animals are infected with street strains of rabies virus by inoculation into subcutaneous tissues or muscle. Clinical disease under natural conditions does not usually develop for a period of weeks to months and sometimes for over 1 year. There is evidence that the virus remains close to the site of inoculation during most of the long incubation period in natural rabies (Charlton *et al*, 1997). In experimental rabies and in studies performed *in vitro*, it has been demonstrated that rabies virus binds to nicotinic acetylcholine receptors at neuromuscular junctions (Lentz *et al*, 1982). We know also that rabies virus spreads within axons by retrograde fast axonal transport.

Although recent evidence has shown that the rabies virus phosphoprotein interacts with the cytoplasmic dynein light chain (Jacob *et al*, 2000; Raux *et al*, 2000), which is an important component of the microtubule-based transport system, it is not clear whether this alone accounts for the viral transport mechanism. After infection develops in spinal cord or brain stem neurons, rabies virus disseminates rapidly throughout the central nervous system by fast axonal transport along neuroanatomical connections. Under natural conditions, rabies virus infection of the CNS causes only relatively mild neuropathological changes without prominent evidence of neuronal death (Iwasaki and Tobita, 2002). Together, these observations have led to the concept that the neurological disease in rabies must result from neuronal dysfunction rather than neuronal cell death. A variety of studies of rabies virus infection in experimental animals and *in vitro* have provided evidence of abnormalities in

neurotransmission involving acetylcholine, serotonin, and γ-amino-n-butyric acid (GABA) (Jackson, 2002). Dysfunction of ion channels has been shown in infected cultured cells (Iwata *et al*, 1999). Induction of inducible nitric oxide synthase mRNA (Koprowski *et al*, 1993) and increased levels of nitric oxide (Hooper *et al*, 1995) have been demonstrated in rabies virus-infected rodents, but the significance of all of these findings in experimental rabies is uncertain. What relevance they may have is also uncertain, because no fundamental defect has yet been found explaining the neuronal dysfunction in natural rabies.

Viruses may cause cell death by either apoptosis or necrosis, and this topic has been recently reviewed (Griffin and Hardwick, 1999; Allsopp and Fazakerley, 2000; Fazakerley and Allsopp, 2001). Apoptosis depends on synthesis of macromolecules and requires energy, while necrosis is associated with energy failure. Each of these forms of cell death is associated with typical morphologic features. The challenge virus strain (CVS) of fixed rabies virus has been observed to induce apoptotic cell death in rat prostatic adenocarcinoma cells (Jackson and Rossiter, 1997), mouse neuroblastoma cells (Theerasurakarn and Ubol, 1998), and mouse embryonic hippocampal neurons (Morimoto *et al*, 1999). Morimoto and coworkers have observed that variants that are more neurovirulent in adult mice produce less apoptosis over a period of 72 h in primary hippocampal neurons than is produced by less neurovirulent variants (Morimoto *et al*, 1999).

Prominent apoptotic death has been observed in neurons in the brains of mice of various ages that were inoculated intracerebrally with the CVS strain and immunosuppression of adult mice did not reduce the apoptotic process (Jackson and Rossiter, 1997; Jackson and Park, 1998; Theerasurakarn and Ubol, 1998). In this issue of the *Journal of NeuroVirology*, Guigoni and Coulon (2002) describe an experimental *in vitro* system in which they studied the capacity of embryonic neurons to survive rabies virus infection. They found that primary cultures of CVS-infected purified rat spinal motoneurons did not show major evidence of apoptosis over a period of 7 days, while infected neurons in crude primary

Address correspondence to Alan C Jackson, Kingston General Hospital, Connell 725, 76 Stuart Street, Kingston, ON, Canada K7L 2V7. E-mail: jacksona@post.queensu.ca

spinal cord cultures did not survive more than 2 days. This difference in survival was not dependent on the presence of factors in the culture medium. In contrast, cultures of purified hippocampal neurons showed apoptosis in over 90% of neurons within 3 days. These results suggest that different neuronal cell types respond differently to rabies virus infection, and that the presence of glial cells and/or neurons other than motoneurons are essential for apoptosis of spinal motoneurons. Physical contact with glia or synaptic contact with other spinal cord neurons may be necessary for induction of apoptosis in motoneurons, but not for apoptosis of hippocampal neurons.

However, apoptosis in infected cells in culture, including embryonic cells, does not closely correspond to what is observed in infected animals. Animals inoculated peripherally with CVS strains do not show the prominent apoptosis that is observed in neurons infected with the same virus after intracerebral inoculation (Reid and Jackson, 2001). Also, conflicting results have been reported by different investigators with respect to the occurrence of neuronal apoptosis after intracerebral inoculation of different street (wild-type) rabies virus variants in mice (Ubol and Kasisith, 2000; Yan *et al*, 2001). Hence, in rabies virus infection it appears there are complex mechanisms involved in cell death or survival of neurons both *in vitro* and in animal models using different viral strains and routes of inoculation. On a more hopeful note, neuronal cell death is not prominent in natural rabies, and perhaps a greater understanding of the mechanisms involved in neuronal apoptosis in experimental animal models may provide further insights into the pathogenesis of neuronal dysfunction that occurs in natural rabies.

In studies using a rabies virus glycoprotein-deficient recombinant rabies virus, Etessami *et al* (2000) recently demonstrated that the glycoprotein is important for the trans-synaptic spread of rabies virus between neurons. Also in this issue of the *Journal of NeuroVirology*, Yan *et al* (2002) examined the role of the rabies virus glycoprotein in determining the topographic distribution of rabies virus infection 7 days after stereotaxic inoculation of virus into the hippocampus of rats using a variety of rabies virus strains and recombinant viruses, including a rabies virus recombinant constructed using the vesicular stomatitis virus glycoprotein. With all of the recombinant viruses, the viral distribution was similar to that of parental viruses from which the glycoprotein was derived. Hence, further evidence is provided that the rabies virus glycoprotein exerts a very important influence on the distribution of rabies virus infection in the nervous system. Mazarakis *et al* (2001) have also recently demonstrated that a rabies virus glycoprotein pseudotyped lentivirus (equine infectious anemia virus)-based vector enhances gene transfer to neurons by facilitating retrograde axonal transport. Hence, a variety of studies emphasize the importance of the rabies virus glycoprotein in the uptake, transport, trans-synaptic spread, and topographic distribution of the infection in the nervous system. We are gradually gaining insights into how rabies virus produces neurologic disease in its hosts. Hopefully, these insights will soon be useful in developing new strategies to alter the outcome of one of the most lethal viral diseases in humans.

References

Allsopp TE, Fazakerley JK (2000). Altruistic cell suicide and the specialized case of the virus-infected nervous system. *Trends Neurosci* **23:** 284–290.

Charlton KM, Nadin-Davis S, Casey GA, Wandeler AI (1997). The long incubation period in rabies: Delayed progression of infection in muscle at the site of exposure. *Acta Neuropathol* **94:** 73–77.

Etessami R, Conzelmann KK, Fadai-Ghotbi B, Natelson B, Tsiang H, Ceccaldi PE (2000). Spread and pathogenic characteristics of a G-deficient rabies virus recombinant: An *in vitro* and *in vivo* study. *J Gen Virol* **81** Pt 9: 2147–2153.

Fazakerley JK, Allsopp TE (2001). Programmed cell death in virus infections of the nervous system. *Curr Top Microbiol Immunol* **253:** 95–119.

Griffin DE, Hardwick JM (1999). Perspective: Virus infections and the death of neurons. *Trends Microbiol* **7:** 155–160.

Guigoni C, Coulon P (2002). Rabies virus is not cytolytic for rat spinal motoneurons *in vitro. J NeuroVirol* **8:** 306–317.

Hooper DC, Ohnishi ST, Kean R, Numagami Y, Dietzschold B, Koprowski H (1995). Local nitric oxide production in viral and autoimmune diseases of the central nervous system. *Proc Natl Acad Sci USA* **92:** 5312–5316.

Iwasaki Y, Tobita M (2002). Pathology. In *Rabies*. Jackson AC, Wunner WH (eds). Academic Press: San Diego, pp 283–306.

Iwata M, Komori S, Unno T, Minamoto N, Ohashi H (1999). Modification of membrane currents in mouse neuroblastoma cells following infection with rabies virus. *Br J Pharmacol* **126:** 1691–1698.

Jackson AC (2002). Pathogenesis. In *Rabies*. Jackson AC, Wunner WH (eds). Academic Press: San Diego, pp 245–282.

Jackson AC, Park H (1998). Apoptotic cell death in experimental rabies in suckling mice. *Acta Neuropathol* **95:** 159–164.

Jackson AC, Rossiter JP (1997). Apoptosis plays an important role in experimental rabies virus infection. *J Virol* **71:** 5603–5607.

Jacob Y, Badrane H, Ceccaldi PE, Tordo N (2000). Cytoplasmic dynein LC8 interacts with lyssavirus phosphoprotein. *J Virol* **74:** 10217–10222.

Koprowski H, Zheng YM, Heber-Katz E, Fraser N, Rorke L, Fu ZF, Hanlon C, Dietzschold B (1993). *In vivo* expression of inducible nitric oxide synthase in experimentally induced neurologic disease. *Proc Natl Acad Sci USA* **90:** 3024–3027.

Lentz TL, Burrage TG, Smith AL, Crick J, Tignor GH (1982). Is the acetylcholine receptor a rabies virus receptor? *Science* **215:** 182–184.

Mazarakis ND, Azzouz M, Rohll JB, Ellard FM, Wilkes FJ, Olsen AL, Carter EE, Barber RD, Baban DF, Kingsman SM, Kingsman AJ, O'Malley K, Mitrophanous KA (2001). Rabies virus glycoprotein pseudotyping of lentiviral vectors enables retrograde axonal transport and access to the nervous system after peripheral delivery. *Hum Mol Genet* **10:** 2109–2121.

Morimoto K, Hooper DC, Spitsin S, Koprowski H, Dietzschold B (1999). Pathogenicity of different rabies virus variants inversely correlates with apoptosis and rabies virus glycoprotein expression in infected primary neuron cultures. *J Virol* **73:** 510–518.

Raux H, Flamand A, Blondel D (2000). Interaction of the rabies virus P protein with the LC8 dynein light chain. *J Virol* **74:** 10212–10216.

Reid JE, Jackson AC (2001). Experimental rabies virus infection in *Artibeus jamaicensis* bats with CVS-24 variants. *J NeuroVirol* **7:** 511–517.

Theerasurakarn S, Ubol S (1998). Apoptosis induction in brain during the fixed strain of rabies virus infection correlates with onset and severity of illness. *J NeuroVirol* **4:** 407–414.

Ubol S, Kasisith J (2000). Reactivation of Nedd-2, a developmentally down-regulated apoptotic gene, in apoptosis induced by a street strain of rabies virus. *J Med Microbiol* **49:** 1043–1046.

Yan X, Mohankumar PS, Dietzschold B, Schnell MJ, Fu ZF (2002). The rabies virus glycoprotein determines the distribution of different rabies virus strains in the brain. *J NeuroVirol* **8:** 345–352.

Yan X, Prosniak M, Curtis MT, Weiss ML, Faber M, Dietzschold B, Fu ZF (2001). Silver-haired bat rabies virus variant does not induce apoptosis in the brain of experimentally infected mice. *J NeuroVirol* **7:** 518–527.