

Infection of Bergmann glia in the cerebellum of a skunk experimentally infected with street rabies virus

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Abstract

Rabies virus is a highly neuronotropic virus and glial cell infection is not prominent in the central nervous system (CNS). Paraffin-embedded tissues from the cerebella of skunks experimentally infected with either a skunk salivary gland isolate of street rabies virus or the challenge virus standard (CVS) strain of fixed rabies virus were examined with immunoperoxidase staining for rabies virus antigen by using an anti-rabies virus nucleocapsid protein monoclonal antibody. A skunk infected with street rabies virus showed prominent infection of Bergmann glia. Although infected Purkinje cells were observed, they usually demonstrated a relatively small amount of antigen in their perikarya. A CVS-infected skunk showed many intensely labeled Purkinje cells and a relatively small number of infected Bergmann glia. These findings indicate that although rabies virus is a highly neuronotropic virus, street rabies virus strains do not always demonstrate strict neuronotropism in the central nervous system.

Résumé

Le virus de la rage est un virus ayant un tropisme marqué pour les cellules nerveuses et l'infection des cellules gliales n'est pas observée de façon évidente au niveau du système nerveux central (SNC). Une épreuve de détection du virus rabique par coloration à l'immunoperoxidase, utilisant un anticorps monoclonal dirigé contre la protéine de la nucléocapside du virus de la rage, fut effectuée sur des tissus enrobés de paraffine provenant du cerveau de mofettes infectées expérimentalement, soit avec une souche sauvage du virus rabique provenant des glandes salivaires d'une mofette ou avec la souche virale défi standard (CVS). Chez une mofette infectée avec la souche sauvage du virus, une infection évidente des cellules gliales de Bergmann a été démontrée. Bien que des cellules de Purkinje infectées aient été observées, seulement une faible quantité d'antigène a pu être démontrée dans la région périnucléaire. Chez une mofette infectée avec la souche CVS, l'observation des tissus a permis de détecter de nombreuses cellules de Purkinje colorées de façon marquée et un nombre relativement faible de cellules gliales de Bergmann infectées. Ces résultats indiquent que malgré le tropisme marqué du virus de la rage pour les cellules nerveuses, les souches sauvages du virus rabique ne manifestent pas toujours un tropisme strict pour les cellules nerveuses du CNS.

(Traduit par docteur Serge Messier)

Rabies virus is a highly neuronotropic virus affecting the central nervous system (CNS) of humans and animals that normally causes a severe and fatal encephalomyelitis (1). Negri bodies and rabies virus antigen are frequently observed in the cytoplasm of many neuronal cell types in the CNS. Although infection of astrocytes and oligodendrocytes has occasionally been noted in rabies by using immunohistochemical (2,3) and ultrastructural studies (4–6), non-neuronal infection is not prominent in the CNS. Significant alterations in the cellular tropism of street (wild-type) rabies virus strains in the CNS have not been recognized. Skunks are important vectors of rabies in North America (7). Since the morphologic identification of specific neural cell types is relatively straightforward in the cerebellar cortex, we have examined the cellular tropism of a skunk isolate of street rabies virus in the cerebellar cortex of a skunk and compared the findings with a skunk infected with the challenge virus standard (CVS) of fixed rabies virus.

A striped skunk (*Mephitis mephitis*) was inoculated intramuscularly in the right abductor digiti quinti muscle with 0.03 mL of a 10% salivary gland suspension from a naturally infected skunk (Canadian arctic strain of street rabies virus) with a titer of $10^{7.5}$ TCID₅₀/mL on BHK-21 cells. Clinical rabies developed after an incubation period of 50 d and the disease lasted for 8 d. Another skunk was infected intranasally with 0.5 mL of CVS with a titer of $10^{8.5}$ TCID₅₀/mL on BHK-21 cells, which was deposited by drops into the external nares. Clinical rabies developed after an incubation period of 7 d and the disease lasted for only 1 d. Brains were fixed in 10% buffered formalin (pH 5.3) for 24 h, dehydrated, and embedded in paraffin. The paraffin blocks were obtained from the late Dr. K.M. Charlton (Animal Diseases Research Institute, Nepean, Ontario) in 1988. Immunoperoxidase staining for rabies virus antigen was performed on sections (6 µm) of cerebellum, as previously described (8), by using anti-rabies virus nucleocapsid protein monoclonal antibody

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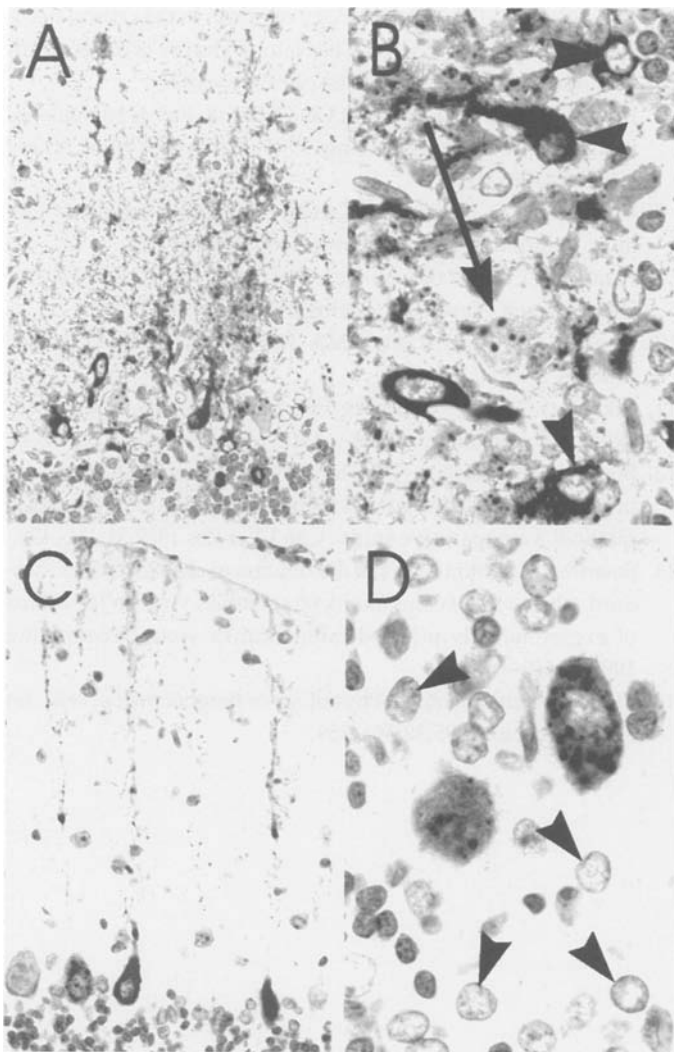


Figure 1. A. Rabies virus antigen in the cerebellum of a skunk infected with a salivary gland isolate of street rabies virus. Multiple Bergmann' glia demonstrate immunostaining for rabies virus antigen in their cell bodies and in cell processes that extend into the molecular layer. B. Heavy antigen staining is seen in the cell bodies of Bergmann glia (arrowheads) and also multiple small foci of antigen are present in the perikaryon of a Purkinje cell (arrow). C. Rabies virus antigen in the cerebellum of a skunk infected with CVS. Antigen is present in the perikarya of three Purkinje cells and in their dendritic processes that extend into the molecular layer to the pial surface. D. Diffuse and multifocal antigen staining is present in the perikarya of two Purkinje cells, whereas nearby Bergmann glia (arrowheads) do not demonstrate staining. Immunoperoxidase-hematoxylin. A: 320X; B: 840X; C: 370X; D: 1110X.

5DF12 (obtained from Dr. Alexander I. Wandeler, Animal Diseases Research Unit, Nepean, Ontario). Controls included immunoperoxidase staining on an uninfected skunk's brain and also on the infected tissues with omission of primary antibody. Cerebellar neural cell types were identified using standard morphologic criteria (9). The numbers of infected and uninfected Purkinje cells and Bergmann glial cells were counted using light microscopy under high magnification (1000 of each for each strain). Infected Purkinje cells were also assessed ($n = 500$) to determine if the area of staining involved $< 20\%$ or $> 80\%$ of the perikaryal area.

In the present study, the skunk isolate of street rabies virus produced prominent infection of Bergmann glia in the cerebellum.

Strong diffuse rabies virus antigen immunoreactivity was observed in the cytoplasm of groups of Bergmann glia and also in their processes in the molecular layer of the cerebellum (Figure 1A, B). Fifteen percent of Bergmann glia demonstrated staining with rabies virus antigen, while 31% of Purkinje cells demonstrated positive staining. Although Purkinje cells frequently showed multiple small, punctate foci of staining for rabies virus antigen in their perikarya, the foci were relatively small and staining was frequently absent in their dendritic processes in the molecular layer. Ninety-two percent of these infected Purkinje cells showed staining involving $< 20\%$ of the perikaryal area, while only 4% of infected Purkinje cells demonstrated staining involving $> 80\%$ of the perikaryal area. Infection was also noted in granule cells in the internal granular layer of the cerebellum and in scattered neurons in the molecular layer. There was an associated inflammatory reaction with perivascular cuffing and infiltration of the leptomeninges and parenchyma with mononuclear inflammatory cells and activated microglia.

The CVS-infected skunk demonstrated prominent infection of Purkinje cells with moderate to intense staining for rabies virus antigen in perikarya and dendritic processes extending into the molecular layer (Figure 1C, D). Thirty-five percent of Purkinje cells demonstrated staining with rabies virus antigen. Of these, 93% demonstrated staining involving $> 80\%$ of the perikaryal area. Rabies virus antigen staining was observed in only 2% of the Bergmann glia, and the amount of staining in these cells was much less than in the street virus-infected skunk. Infection was also noted in granule cells in the internal granular layer of the cerebellum. Inflammatory changes were noted that were similar to the findings in the street virus-infected skunk.

Although non-neuronal infection is well recognized outside of the nervous system in rabies, particularly involving the salivary glands (1), there have been only infrequent reports demonstrating infection of astrocytes or oligodendrocytes in the CNS (2-6). In this study, prominent infection of Bergmann glia was observed in the cerebellum of a skunk infected with street rabies virus, while Purkinje cells demonstrated relatively small amounts of rabies virus antigen staining in their perikarya. These findings are similar to those in the cerebella of mice infected with a skunk strain of rabies virus (A.C. Jackson, unpublished observations). Unfortunately, detailed neuropathologic studies with immunostaining for rabies virus antigen have not been reported on a human case of rabies transmitted by a skunk (10,11). It would be of interest to determine if the cellular tropism of the virus in humans is similar to our findings in a skunk.

Infection of Bergmann glia has not been noted by previous investigators in humans (2), skunks (12,13), or other animal species (3,12). Bergmann glial infection was much less marked and Purkinje cell infection was much more prominent in a CVS-infected skunk. These findings suggest that the strong tropism for Bergmann glia in the street virus-infected skunk was more influenced by the street rabies virus strain than by the species of the host. The glial cell infection observed in the street rabies virus-infected skunk indicates that strict neuronotropism is not always the case in the CNS in rabies. Although it is unclear whether glial infection plays any significant role in the pathogenesis of rabies, infection of glia could cause a variety of derangements in CNS function, including impaired synaptic transmission (14).

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