

SHORT ORIGINAL COMMUNICATION

Alan C. Jackson · Hannah Park

Experimental rabies virus infection of p75^{NTR} neurotrophin receptor-deficient mice

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Abstract The low-affinity neurotrophin (NT) receptor, p75^{NTR}, has complex biologic functions. A recent report provided evidence that the p75^{NTR} is a rabies virus receptor in cultured BSR cells. We studied the experimental infection of 6-day-old p75^{NTR}-deficient mice with the challenge virus standard strain of fixed rabies virus inoculated intracerebrally. The mice developed a fatal encephalitis. There were morphologic changes of apoptotic cell death involving neurons in widespread areas of the brain, which were associated with *in situ* evidence of oligonucleosomal DNA fragmentation. The findings were very similar to those that we previously reported in wild-type ICR mice of the same age. If the p75^{NTR} is an important receptor of rabies virus in animal hosts, then a greater effect on the clinical and pathologic features of rabies virus-infected p75^{NTR}-deficient mice would have been expected.

Key words Apoptosis · Encephalitis · Rabies · Pathogenesis

Introduction

Tuffereau et al. [14] recently reported that the low-affinity neurotrophin receptor, p75^{NTR}, is a rabies virus receptor. p75^{NTR} functions include ligand-binding specificity for nerve growth factor, cooperation with the TrkA tyrosine kinase receptor to form a high-affinity binding site, and

modulation of Trk signaling [2]. p75^{NTR} has been recognized to have a pro-apoptotic role *in vivo* [10], and the absence of p75^{NTR} is neuroprotective during development [15]. However, the role of p75^{NTR} in cell death varies in different systems, and this may be largely related to interactions of p75^{NTR} with the Trks [1]. Tuffereau et al. [14] found that cultured BSR cells expressing p75^{NTR} were permissive for a street rabies virus isolate from a fox. In addition, the p75^{NTR}-expressing BSR cells were found to be three to ten times more susceptible to infection with the challenge virus standard (CVS) strain of fixed rabies virus than control BSR cells (lacking p75^{NTR}) in the presence of 10% serum. We have postulated that if p75^{NTR} is a biologically important receptor for rabies virus in the central nervous system (CNS), then there would likely be a significant effect on the pathogenicity of rabies virus infection of p75^{NTR}-deficient mice. Reduced neurovirulence with altered and/or less severe neuropathologic changes in these mice would be anticipated. We studied CVS infection of suckling p75^{NTR}-deficient mice.

Materials and methods

Six-day-old p75^{NTR}-deficient mice [9] were inoculated intracerebrally with a viral dose of 840 PFU of CVS-11 in 0.02 ml of PBS with 2% fetal bovine serum. Mock-infected mice received the same volume of diluent inoculated intracerebrally. During an observation period of 4 days post-inoculation (p.i.), CVS-infected mice developed clinical rabies with progressively increasing growth retardation, ataxia, and paresis and the mice were moribund by day 4 p.i. Mice were anesthetized with methoxyflurane by inhalation and perfused via the heart with 4% buffered paraformaldehyde. Brains were removed daily from three to four mice between day 2 and day 4 p.i. The brains were subsequently immersed overnight in the same fixative, dehydrated, and embedded in paraffin. Coronal sections of brain and transverse sections of the brain stem were cut on a microtome. Tissue sections were stained for morphologic examination with cresyl violet. Immunoperoxidase staining by the avidin-biotin-peroxidase complex (ABC) method was used for the detection of rabies virus antigen using polyclonal rabbit anti-rabies virus serum as described previously [7]. Evidence of oligonucleosomal DNA fragmentation was evaluated using the terminal deoxynucleotidyl transferase-mediated dUTP-digoxigenin nick end-labeling (TUNEL) method as previously described [6].

A. C. Jackson
Department of Medicine, Queen's University,
Kingston, Ontario, Canada

A. C. Jackson · H. Park
Department of Microbiology and Immunology,
Queen's University, Kingston, Ontario, Canada

A. C. Jackson (✉)
Kingston General Hospital, Connell 725, 76 Stuart St.,
Kingston, Ontario, Canada K7L 2V7
e-mail: jacksona@post.queensu.ca
Tel.: +1-613-548-1316, Fax: +1-613-548-1317

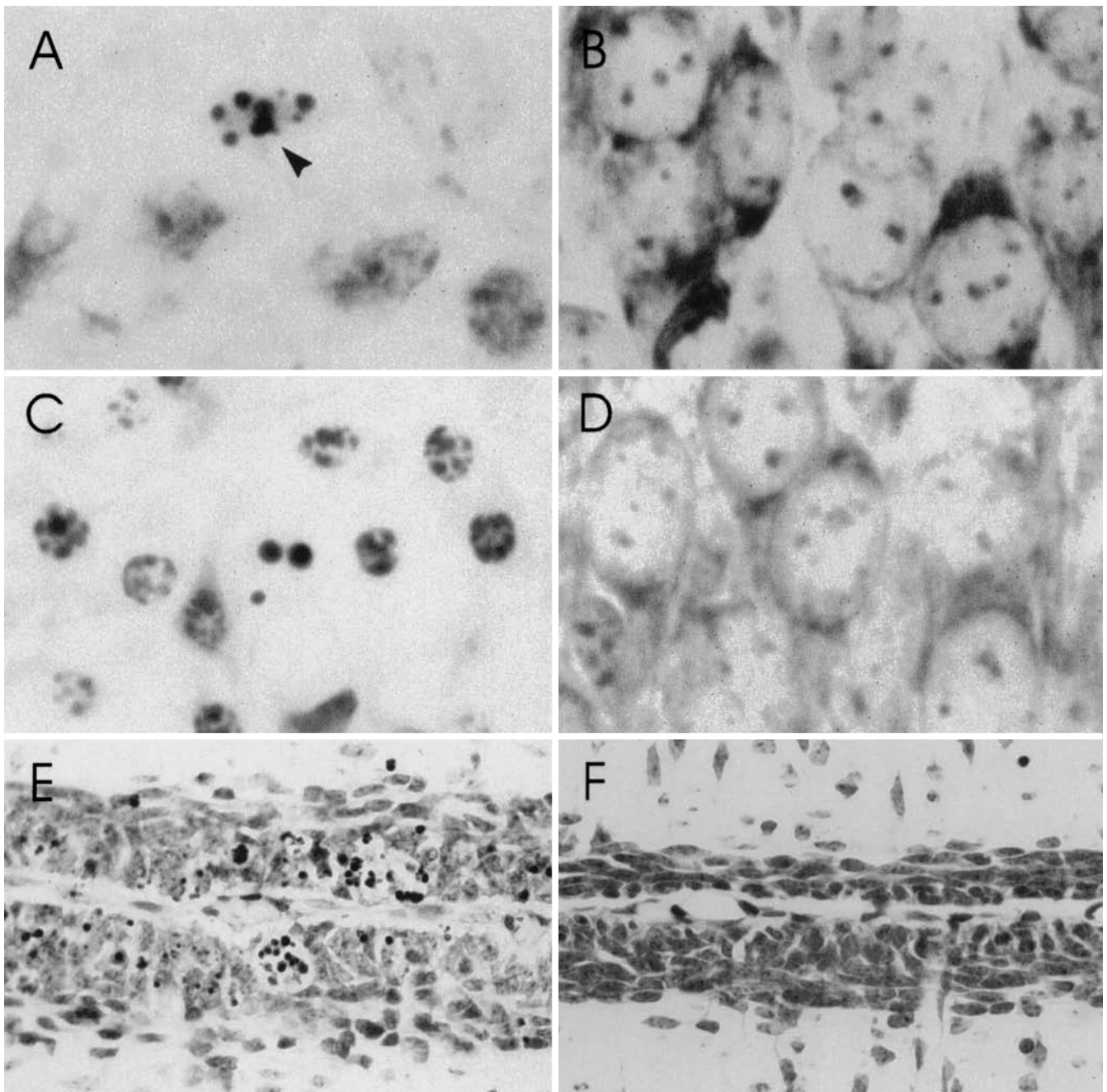


Fig. 1 A Pyriform cortex 4 days p.i. with CVS showing multiple condensations of nuclear chromatin in a large cell that is likely a neuron (*arrowhead*). B Mock-infected mouse showing normal neurons in the pyriform cortex. C Pyramidal neurons of the hippocampus (CA1 region) 3 days p.i. with CVS showing multiple condensations of nuclear chromatin. D Mock-infected mouse showing normal pyramidal neurons of the hippocampus (CA1 region). E External granular layers of the cerebellum 4 days p.i. with CVS showing many cells with nuclear chromatin condensations. F In contrast, a mock-infected mouse demonstrates relatively few cells in the external granular layer and in the adjacent molecular layer of the cerebellum with nuclear chromatin condensations (CVS challenge virus standard, *p.i.* post-inoculation). A–F Cresyl violet staining. A, B $\times 2020$; C, D $\times 1620$; E, F $\times 460$

Results

Widespread apoptotic changes were observed in the brains of infected p75^{NTR}-deficient mice, which were similar to the pathologic changes observed in a variety of mouse models of experimental rabies [4, 5, 12]. Morphologic features of neuronal apoptosis, including condensations of nuclear chromatin and cytoplasmic shrinkage, were present on day 2 p.i. and they became progressively more severe on days 3 and 4 p.i. Severe changes were noted in neurons in the cerebral cortex and pyramidal neurons of the hippocampus (Figs. 1A, C; 2A). Moderately severe changes of apoptosis were observed in neurons in

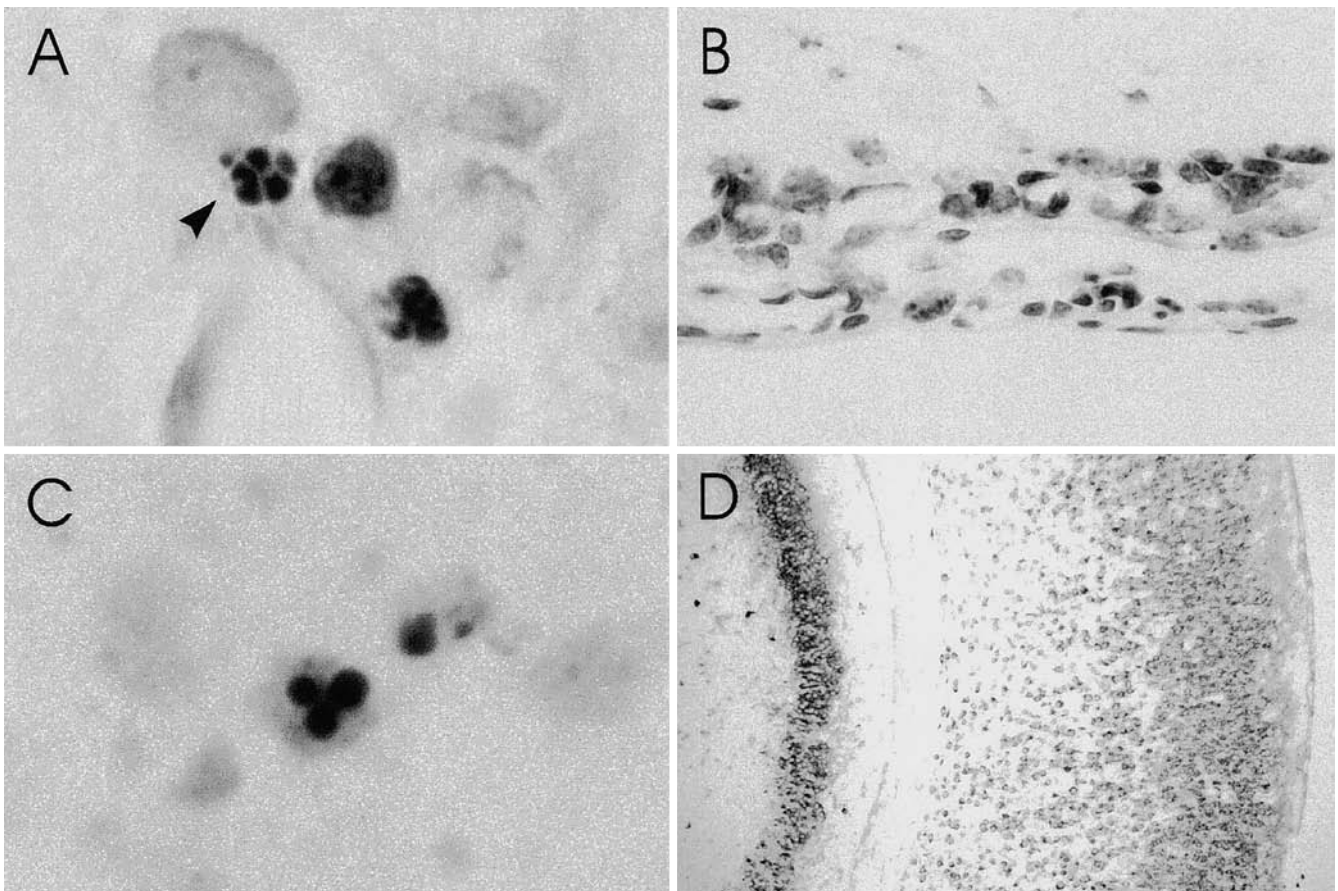


Fig. 2 **A** Condensation of nuclear chromatin of neurons in the cerebral cortex, including one showing nuclear fragmentation (*arrowhead*), 3 days p.i. with CVS. Cresyl violet staining. **B** Infiltration of the leptomeninges with mononuclear inflammatory cells 4 days p.i. with CVS. Cresyl violet staining. **C** TUNEL staining of nuclei of large cells that are likely neurons in the cerebral cortex 4 days p.i. with CVS showing evidence of oligonucleosomal DNA fragmentation in the cells. **D** Immunoperoxidase staining for rabies virus antigen of a mouse 4 days p.i. with CVS showing antigen in many pyramidal neurons of the hippocampus (*left*) and many neurons in all layers of the cerebral cortex (*right*), indicating widespread infection. **A** $\times 2290$; **B** $\times 570$; **C** $\times 1550$; **D** $\times 65$

the medial septal region, dentate gyrus of the hippocampus, diencephalon, brain stem, and external layer of the cerebellum (Fig. 1E). In contrast, mock-infected mice demonstrated relatively few neurons in the brain with apoptotic changes (Fig. 1B, D, F). There was infiltration with mononuclear inflammatory cells in the leptomeninges (Fig. 2B), in a perivascular distribution, and in the brain parenchyma. The inflammatory changes were more marked at the late time points.

TUNEL staining was observed in cells with features of neurons in the above regions on day 3 p.i. and increased staining was present on day 4 p.i. (Fig. 2C). Cells with positive TUNEL staining were observed in the same regions as neurons with morphologic changes of apoptosis. There was a widespread distribution of rabies virus antigen in the brains of infected mice on day 2, and the number of infected neurons subsequently increased on days 3

and 4 (Fig. 2D). As noted previously in ICR mice [4], all neurons containing rabies virus antigen (e.g., Purkinje cells) did not demonstrate apoptotic changes or TUNEL staining, and neurons undergoing apoptosis in the external granular layer of the cerebellum did not demonstrate staining for rabies virus antigen.

Discussion

The CVS strain of rabies virus is able to attach, penetrate, and replicate in a variety of mammalian cell lines, while street rabies virus strains are unable to infect most cell lines except neuroblastoma cells [11, 14]. Although there are ubiquitous receptors for fixed strains of rabies virus on cultured cells, the population of rabies virus receptors is much more restricted in vivo in neural cells as demonstrated by the neuronotropism of the fixed strains in the CNS [3]. In this study, rabies virus infection of p75^{NTR}-deficient mice produced a severe and fatal encephalitis, which was indistinguishable from the encephalitis in wild-type mice. Pathologically, there were marked changes with morphologic features of apoptosis in widespread brain areas, which were similar to the findings that we reported previously in infected ICR mice of the same age [4]. Positive TUNEL staining provided evidence of DNA fragmentation that is typical of the apoptotic process. If the p75^{NTR} is an important and essential receptor for rabies

virus in the CNS, then one would have expected significant alterations in the clinical disease and/or neuropathologic features of the disease in the brain of these knockout mice. Since ubiquitous receptors for fixed rabies strains are not present on neural cells in the CNS, it is unlikely that p75^{NTR} is an important receptor for animals infected with street rabies virus, but not CVS. However, this would need to be examined in p75^{NTR}-deficient mice with a street rabies virus isolate. Jackson et al. [8] observed that spontaneous recovery occurs in adult ICR mice infected peripherally with a fox isolate of street rabies virus, which might complicate assessment of street virus infection of p75^{NTR}-deficient mice. In conclusion, either p75^{NTR} may not be an important rabies virus receptor in the mouse brain or the virus may have the ability to also efficiently utilize other receptors in brain neurons in this experimental mouse of rabies, and there is recent evidence that the neural cell adhesion molecule is also a receptor for rabies virus [13].

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