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Comparative pathogenesis of the SAD-L16 strain of rabies virus and a mutant modifying the dynein light chain binding site of the rabies virus phosphoprotein in young mice

Pamini Rasalingam[a], John P. Rossiter[b], Teshome Mebatsion[c], Alan C. Jackson[a,d,*]

[a] *Department of Microbiology and Immunology, Queen's University, Kingston, Ont., Canada*
[b] *Department of Pathology and Molecular Medicine, Queen's University, Kingston, Ont., Canada*
[c] *Research and Development, Intervet Inc., Millsboro, DE, USA*
[d] *Department of Medicine (Neurology), Queen's University, Kingston, Ont., Canada*

Abstract

Recent reports have suggested that rabies virus phosphoprotein (P) interaction with dynein minus-end-directed microtubule motor proteins may be of fundamental importance in the axonal transport of rabies virus. A deletion of 11 amino acids was introduced into recombinant rabies virus SAD-L16 (L16) that modified the dynein light chain (LC8) binding site of the rabies virus P, producing mutant L-ΔP11. This mutant is a useful tool for determining the role of P–LC8 interaction in viral spread and pathogenesis. Seven-day-old ICR mice were inoculated into a hindlimb thigh muscle with L16 or L-ΔP11. Histopathological and immunohistochemical analyses of their brains were performed at serial time points in order to determine the pattern of viral spread. L16 spread to the brain and caused a severe encephalitis with apoptotic neuronal changes. L-ΔP11 infected specific brain areas (brainstem and hippocampus) 1–2 days later than L16 and involved a smaller number of neurons in some brain regions. However, the neuronal apoptotic changes produced by both viruses were similar in most brain regions. Following peripheral inoculation, deletions modifying the LC8 binding site had an effect on delaying viral spread, but did not significantly alter the pattern of rabies virus encephalitis. The precise role of the rabies virus P–dynein interaction in the axonal transport of rabies virus, particularly the importance of this interaction during natural infection, merits further study.

Keywords: Neurovirulence; Pathogenesis; Rabies; Virulence

1. Introduction

Rabies virus is a highly neurotropic virus that produces fatal encephalomyelitis in humans and animals (Jackson, 2002b; Jackson and Wunner, 2002; Niezgoda et al., 2002). After peripheral inoculation rabies virus spreads through peripheral nerves to the central nervous system (CNS) by retrograde fast axonal transport (Tsiang, 1979; Gillet et al., 1986; Jackson, 2002a). It has been recognized that a variety of toxins and pathogens disseminate in the nervous system by hijacking cellular transport machinery (Mueller et al., 2001; Von Bartheld, 2004). Cytoplasmic dynein serves an important role in retrograde axonal transport (Vallee et al., 2004). The cytoplasmic dynein light chain (LC8) has recently been shown to interact with the rabies virus P using laser confocal microscopy and immunoprecipitation (Jacob et al., 2000; Raux et al., 2000). Analyses of a series of deletions mapped the LC8-binding domain to residues 138–172 of the P (Raux et al., 2000). LC8 is an important component of both cytoplasmic dynein and myosin V, which are important in microtubule minus-end-directed organelle transport and in actin-based vesicle transport, respectively. Consequently, it has been proposed that the rabies virus P–LC8 interaction might play an important role in the axonal transport of rabies virus along microtubules in neurons (Jacob et al., 2000; Raux et al., 2000). However, since rabies virus is internalized by receptor-mediated endocytosis (Superti et al., 1984),

* Corresponding author. Present address: Kingston General Hospital, Connell 725, 76 Stuart Street Kingston, Ont., K7L 2V7, Canada.
Tel.: +1 613 548 1316; fax: +1 613 548 1317.
E-mail address: jacksona@post.queensu.ca (A.C. Jackson).

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viral uncoating would need to occur shortly after viral entry for rabies virus P–LC8 interaction to take place for initiation of retrograde axonal transport from, for example, axon terminals unless there is an unknown mechanism by which an intact endosome containing rabies virus could attach to the dynein motor protein and be transported along the axon (Schnell et al., 2005). Experimental evidence is lacking that viral uncoating actually occurs at this site, and the actual role of rabies virus P–LC8 interaction in axonal transport remains speculative.

In an effort to develop a new live attenuated rabies vaccine with impaired neuronal transport, Mebatsion (2001) generated recombinant rabies viruses with deletions encompassing a conserved LC8-interacting motif ((K/R)XTQT) in the LC8 binding site (Lo et al., 2001). The deletions blocked incorporation of LC8 into mature virions and completely abolished the P–LC8 interaction. Intriguingly, mortality data at the end of a 21 day observation period indicated that SAD-L16 (L16) and the deletion mutants derived from it were equally virulent in 1-week-old mice after intramuscular inoculation, indicating that LC8 is dispensable for the spread of a pathogenic rabies virus from a peripheral site to the CNS in young mice. However, this mortality data may not reflect differences in virus transport. In the present study, we compare the time course of viral spread and neuropathological changes of a mutant with a deletion of 11 amino acids of the LC8 binding site, L-ΔP11, with parent virus L16 after intramuscular inoculation in 7-day-old mice in order to further assess the biological importance of the LC8 binding site in experimental rabies pathogenesis.

2. Materials and methods

2.1. Viruses

Rabies virus belongs to the *Lyssavirus* genus in the *Rhabdoviridae* family in the *Mononegavirales* order. The generation of recombinant rabies viruses L16 and L-ΔP11 was previously described (Mebatsion, 2001).

2.2. Animals and inoculations

Seven-day-old ICR mice (Charles River Canada, St. Constant, Quebec) were used. Mice were inoculated intramuscularly into the right hindlimb thigh muscle with 20 μL containing 1000 focus-forming units of either L16 or L-ΔP11 diluted in PBS with 4% fetal bovine serum. Uninfected control mice were inoculated with only the diluent.

2.3. Preparation of tissue sections

Two to three mice were killed at daily intervals. Mice were anesthetized with methoxyflurane and perfused with buffered 4% paraformadehyde. Brains were removed and immersion-fixed in the same fixative for 24 h at 4 °C. Coronal brain tissue sections (6 μm) were prepared after dehydration and embedding in paraffin, and were stained with cresyl violet for light microscopic examination.

2.4. Immunoperoxidase staining for rabies virus antigen

Sections were stained for rabies virus antigen by the avidin-biotin-peroxidase complex method using monoclonal mouse anti-rabies virus IgG 5DF12 primary antibody (obtained from Alexander I. Wandeler, Centre of Expertise for Rabies, Canadian Food Inspection Agency, Nepean, Ontario) as previously described (Jackson et al., 1999). Brain sections from uninfected mice were used as controls. In brief, tissue sections were deparaffinized, hydrated, and were successively reacted with 5% normal rabbit serum, monoclonal mouse anti-rabies virus nucleocapsid protein IgG 5DF12 diluted 1:160, biotinylated rabbit anti-mouse IgG secondary antibody, diluted 1:100 (Vector Laboratories, Burlingame, CA), 1% hydrogen peroxide in methanol, avidin-biotinylated horseradish peroxidase complex (Vector Laboratories), 3, 3-diaminobenzidine tetrachloride (Polysciences, Warrington, PA) with 0.01% hydrogen peroxide, 0.5% cupric sulfate in 0.15 M sodium chloride. The sections were counterstained with hematoxylin.

3. Results

3.1. Clinical data

L16-infected mice developed hindlimb paralysis on day 4 post-inoculation (p.i.), by which time there was evidence of growth retardation. Over the next 3 days there was progression to quadriparesis and all surviving mice were moribund by day 8 p.i.

L-ΔP11-infected mice demonstrated hindlimb paralysis 1 day later than L16 (on day 5 p.i.) and also had growth retardation. Over the next 3–6 days the mice progressed to quadriparesis or a moribund state. Some mice survived up to 3 days longer than the L16-infected mice.

3.2. Rabies virus antigen distribution and histopathology

3.2.1. Virus distribution

In L16 infection rabies virus antigen was first detected in brainstem neurons at 4 days p.i. By day 6 p.i. L16 had spread to involve the thalamus, deep cerebellar nuclei (Fig. 1A), Purkinje cells in the cerebellar cortex, cerebral cortex, and pyramidal neurons of the hippocampus. The infection in the cerebral cortex was usually prominent before significant involvement of hippocampal pyramidal neurons (Fig. 2A). Many hippocampal pyramidal neurons were infected on day 7 or 8 (Fig. 2C). Antigen was not observed in the dentate gyrus of the hippocampal formation or in the internal or external granular cell layers of the cerebellum.