

Overlap of Pathology in Paralytic Rabies and Axonal Guillain–Barré Syndrome

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We describe clinical and pathological features of a case of paralytic rabies with acute axonal neuropathy that closely resembled axonal Guillain–Barré syndrome. This case emphasizes that there is overlap of both clinical and pathological features in paralytic rabies and axonal Guillain–Barré syndrome. These findings raise the possibility that infectious and autoimmune etiologies can lead to similar morphological changes in the nerves.

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In 1997, some of us (J.W.G., A.K.A., C.Y.L.) with G. M. McKhann and T. W. Ho described four cases of the acute axonal motor sensory (AMSAN) form of Guillain–Barré syndrome (GBS).¹ Further studies, which are described below, have indicated that Case 1 was actually the paralytic form of rabies. Recently, M.R.-A., who has had extensive experience with the neuropathology seen in fatal cases of acute flaccid paralysis, including those with GBS, polio, and paralytic rabies reviewed our cases from China. M.R.-A. suggested that cytoplasmic changes in the anterior horn cells of this case were consistent with the paralytic form of rabies. This study shows how this diagnosis was confirmed. In our case of paralytic rabies, axonal degeneration in ventral spinal roots and peripheral nerves was the most prominent pathological finding. We suggest that the pathological spectrum of paralytic rabies includes acute axonal neuropathy without overt neuronopathy or inflammation, and, in the absence of history of exposure, such cases may be indistinguishable

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from axonal forms of GBS by pathology and/or electrophysiology.

Case Report

The patient, a 55-year-old male resident of Hebei Province, China, developed abrupt left arm weakness that progressed over 4 days to tetraparesis with difficulties in swallowing and respiration requiring assisted ventilation. The patient died 7 days after the onset of weakness. Diarrhea had been present 15 days before the onset of neurological symptoms. There was a history of dog bite and rabies vaccination 6 years before this presentation. There was no history of another exposure to a rabid animal. The patient displayed no sensory disturbance or typical features of rabies encephalitis, including hydrophobia during his final illness.

Electrophysiological studies showed decreased compound muscle action potential amplitudes, normal sensory nerve action potential amplitudes, and normal motor and sensory conduction velocities. Blood was drawn for serology. Cerebrospinal fluid was not examined. An autopsy was performed within 2 hours of death; tissues collected included thoracic and lumbar spinal cord and ventral and dorsal roots, ulnar and median nerves, and the sciatic nerve below the sciatic notch. Brain and cervical spinal cord were not obtained.

Methods

SEROLOGY. Antibodies directed against *Campylobacter jejuni* and gangliosides asialo-GM1 (GA1), GM1, GD1a, GD1b, and GQ1b were measured as described previously.² Neutralizing antirabies virus antibodies were measured by the rapid fluorescent focus inhibition test³ kindly performed by Dr. C. Rupprecht (CDC, Atlanta, GA).

PATHOLOGY. Postmortem tissue was fixed in paraformaldehyde or glutaraldehyde. Paraformaldehyde-fixed tissue samples were embedded in paraffin and sections were stained with hematoxylin and eosin and a combined silver–Luxol fast blue–periodic acid Schiff stain. Teased fibers were prepared from spinal roots. The glutaraldehyde-fixed samples were osmicated, embedded in epoxy resin, and examined by light (toluidine blue–stained 1 μ m sections) and electron microscopy.

IMMUNOPATHOLOGY. Paraffin sections of spinal cord and roots were immunostained for human macrophage and microglial marker (HAM56), lymphocyte common antigen, the major histocompatibility complex class II (HLA-DR), complement activation markers (C3d and C5b-9), human IgG, and rabies viral nucleocapsid antigen as described.^{4,5} Spinal cord and roots from axonal and demyelinating GBS cases (Chinese Cases 11 and 12) were used as controls for rabies virus nucleocapsid staining. Sural nerve biopsies with Wallerian-like degeneration and spinal cords from normal controls and GBS patients were used as controls for human IgG and complement immunostaining.

IN SITU HYBRIDIZATION. In situ hybridization was performed on paraffin-embedded spinal cord tissue from the index case and uninfected normal controls using specific single-stranded RNA probes for rabies virus genomic RNA as previously described.⁶

Results

Serology

Enzyme-linked immunosorbent assay was positive for anti-*C. jejuni* antibodies and negative for IgG and IgM antiganglioside antibodies. Rapid fluorescent focus inhibition test assay showed the presence of antirabies virus antibodies with a titer of 1 to 5.

Pathology and Immunopathology

In spinal cord sections, motor neurons had only mild chromatolytic changes and their number appeared normal. There was no neuronal phagocytosis or degenera-

tion of spinal cord white matter (Fig 1A). There was clumping of the Nissl granules in motor neurons on hematoxylin and eosin sections, with a characteristic patchiness on Bodian silver, in which the faintly stained areas presumably represent the blocks of Nissl (see Fig 1A, B). Neither Negri bodies nor Lyssa bodies were identified. Rabies virus genomic RNA was detected in perikarya of anterior horn cells by in situ hybridization (see Fig 1B). Immunostaining in spinal cord showed the presence of rabies virus nucleocapsid antigen in the neuronal perikarya, dendrites, and axons in the anterior horns (see Fig 1C), some dorsal horn neurons, predominantly axons in the anterolateral columns, and some microglia in both gray and white matter. No staining was seen in normal or GBS controls. Immunostaining showed absence of T-cell inflammation or macrophage recruitment. Activated microglia

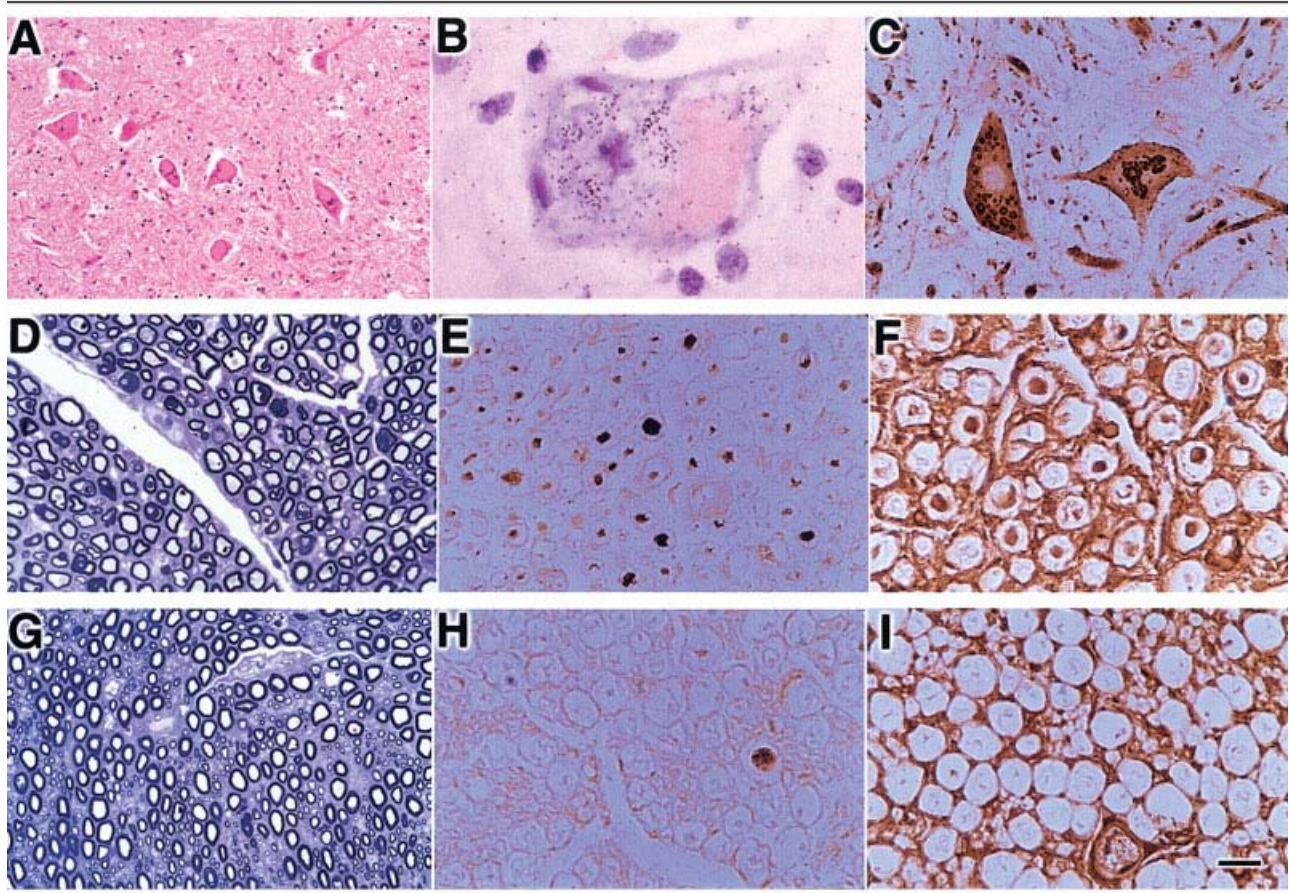


Fig 1. (A–C) Paraffin sections of lumbar spinal cord. (A) Hematoxylin and eosin stain showing alteration of Nissl staining in some ventral horn neurons and lack of inflammation. (B) In situ hybridization with a ³H-labeled RNA probe showing rabies virus genomic RNA in the perikaryon of an anterior horn cell. (C) Immunoperoxidase staining showing rabies virus nucleocapsid antigen in multiple anterior horn cells and their dendritic processes. (D–F) Ventral root. (D) Plastic section (1µm) stained with toluidine blue showing axonal degeneration. (E) Rabies nucleocapsid antigen is present in a large proportion of myelinated axons. (F) Human IgG is deposited on some myelinated axons. (G–I) Dorsal root. (G) Plastic section (1µm) stained with toluidine blue showing relatively preserved morphology. (H) Rabies virus nucleocapsid antigen is present in an occasional axon. (I) In contrast with ventral root, IgG is not deposited on dorsal root axons. Bar = 10µm.

(HLA-DR positive) were present in ventral horn white matter tracts and dorsal horns. IgG and complement staining in the index case was not above the level in controls.

Spinal Roots

The toluidine blue-stained 1 μ m sections showed that Wallerian-like degeneration was much more severe in ventral than in dorsal spinal roots (see Fig 1D, G). These findings were also confirmed by teased fiber preparations. Internodal demyelination was observed in occasional fibers (<1%). Immunostaining showed scanty T-cell inflammation in the roots. The macrophage and HLA-DR staining correlated with the extent of Wallerian degeneration, being more prominent in ventral than in dorsal roots. In contrast with spinal roots, only occasional and very early Wallerian-like degeneration was seen in peripheral nerves.

Many myelinated axons in the ventral roots and occasional fibers in the dorsal roots were positive for viral nucleocapsid antigen (see Fig 1E, H). No staining was seen in control nerves. Similar to the distribution of viral antigens, a high number of myelinated axons in

ventral roots were positive for human IgG (see Fig 1F), whereas only rare fibers in dorsal roots were positive (see Fig 1I). Double-labeling experiments confirmed colocalization of human IgG and rabies viral antigens on axons from the ventral roots (Fig 2A–C). Colocalization of human IgG and C3d on ventral root axons was also seen (see Fig 2D–F). C5b-9 staining was much less robust than C3d. Sural nerves from controls lacked such staining.

Electron Microscopy

Some lumbar motor neurons showed clearing of rough endoplasmic reticulum from areas of the motor neuron cell bodies, which contained tightly packed skeins of neurofilaments and clumping of endoplasmic reticulum in other areas of the same perikaryon (Fig 3A). The motor neuron soma contained viral matrices identified by bullet-shaped mature viral particles in the soma of some lumbar motor neurons; viral profiles surrounded by amorphous electron-dense material (see Fig 3A, B). In ventral roots, macrophages were prominent within the periaxonal space of many myelinated fibers, around both apparently intact and degenerating axons as de-

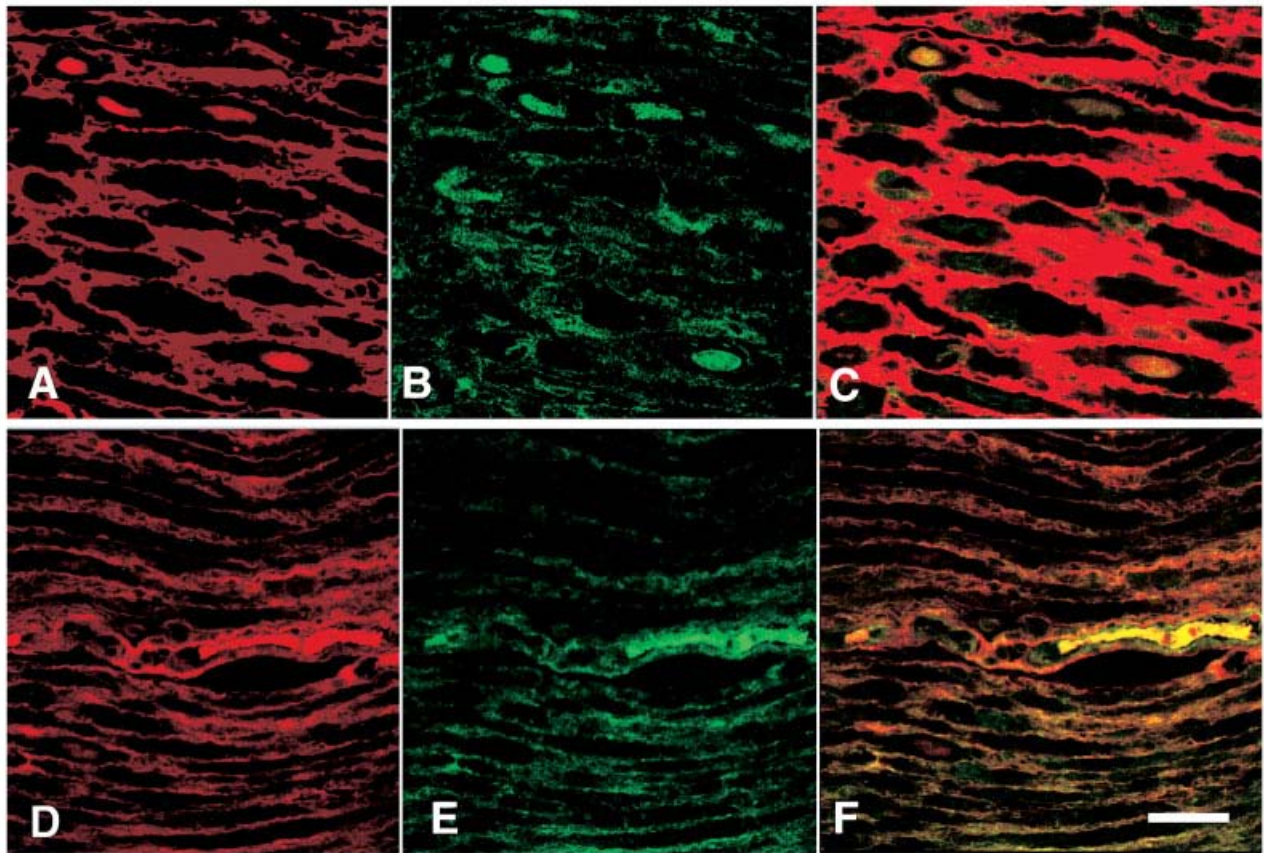


Fig 2. Confocal images from ventral root. (A–C) Axons showing immunostaining for human IgG (red) (A), rabies virus nucleocapsid antigen (green) (B), and colocalization (C). (D–F) Axons showing immunostaining for human IgG (red) (D), complement C3d (green) (E), and colocalization (F). Bar = 4 μ m.

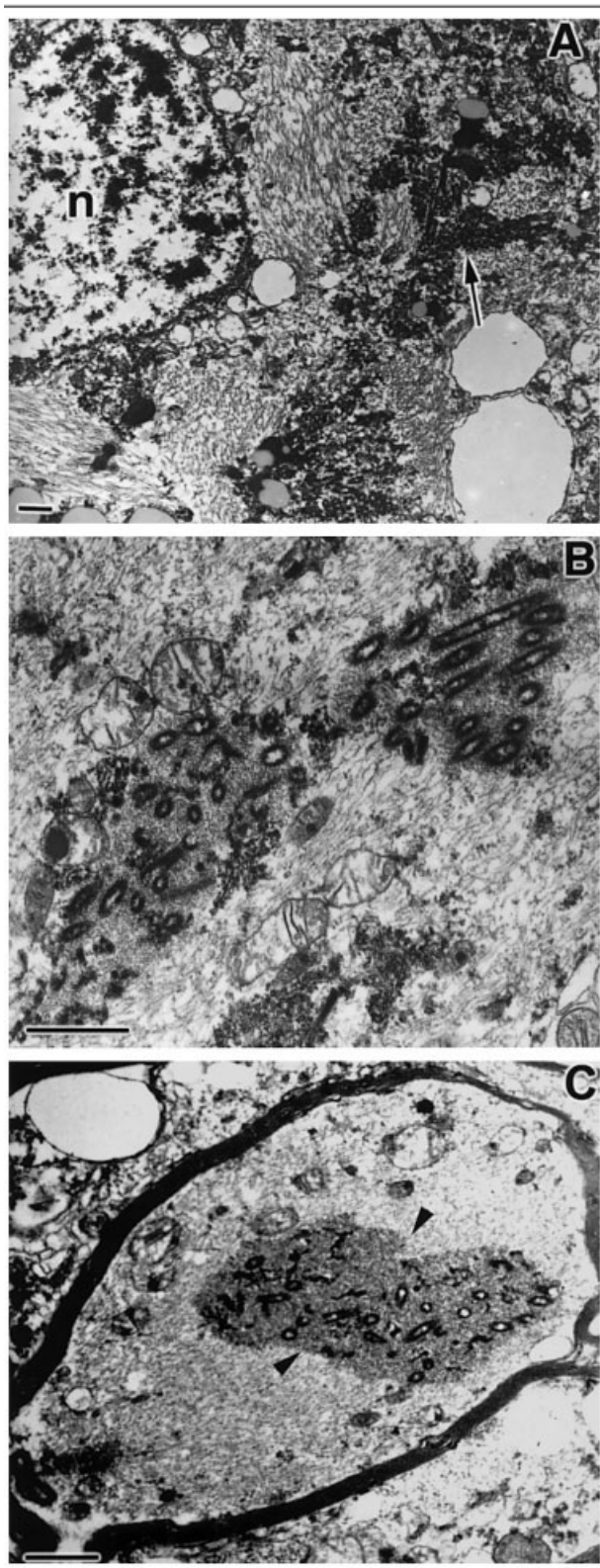


Fig 3. Electron micrographs from spinal cord ventral horn. (A) Ventral horn neuron infected with rabies virions (arrow) dispersed between tightly packed neurofilaments; n = nucleus. (B) High-power view of a viral matrix; bullet-shaped virions are embedded in a proteinaceous material. (C) Axon containing a rabies viral matrix (arrowheads). Bar = 1 μ m.

scribed previously.¹ Some myelinated axonal profiles in ventral root exit zone contained bullet-shaped mature viral particles (see Fig 3C).

Discussion

GBS-like presentations of rabies with paralytic features have been recognized for many years.⁷ This case illustrates that acute axonal neuropathy can be seen in paralytic rabies, and distinction between such cases of paralytic rabies and axonal GBS cannot be made solely by clinical, electrophysiological, and pathological criteria. In endemic areas, paralytic rabies should always be included in the differential diagnosis of acute flaccid paralysis, which may also be caused by other neurotropic viruses.⁸

Our case initially was confused with GBS because of history of diarrhea before presentation, lack of typical features of rabies such as phobic spasms, and a history of dog bite and antirabies vaccination 6 years before the paralytic illness. Although previously unvaccinated cases with long incubation periods have been described previously,⁹ we believe that it is more likely that rabies virus was transmitted to this patient as a result of another exposure to rabies virus that was unrecognized, forgotten, or not communicated to his family.

Our case emphasizes that predominant motor axonal neuropathy in the absence of prominent inflammation or motor neuron degeneration may be the only neuropathological finding in some cases with paralytic rabies and represents one end of the pathological spectrum of this disease. Previous reports have indicated large variations in the amount of inflammation and neuroanatomic changes in the spinal cord and demyelinating and axonal injury in peripheral nerves of cases with paralytic rabies.^{10,11} The pathogenetic basis of paralysis in rabies remains unclear.¹² One possibility raised by this case is that axonal (neuronal process) degeneration could be the first morphological consequence of rabies virus infection of neuronal perikarya. That this pathogenetic sequence may not be unique to infections like rabies, but could also be seen in neurodegenerative disorders, such as amyotrophic lateral sclerosis, is reported in a recent study that showed that motor axon degeneration can precede neuronal loss in an animal model of amyotrophic lateral sclerosis.¹³

Alternatively, it is possible that axonal degeneration may be caused by immune injury because we found immunoglobulins and activated complement were deposited on the axons. A recent report has proposed such a pathogenetic sequence in a case of encephalitic rabies complicated by paralysis after treatment with intravenous rabies immune globulin.¹⁴ The absence of antirabies virus antibodies in some paralytic cases would argue that antibody-mediated injury is one possible mechanism in some but not all cases that can contribute to the complex pathophysiology of this dis-

ease.^{12,15,16} Reproduction of key pathological features seen in this case and axonal GBS in an animal model, induced by immunization with gangliosides,^{17,18} would support the hypotheses that the pathological changes in axonal GBS are not related to a viral infection of the nervous system but to antibody-mediated axonal injury.

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