Neuromyelitis Optica With Extensive Active Brain Involvement

An Autopsy Study

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Objective: To describe the clinical, molecular, and neuropathological findings of a patient with aquaporin 4–positive relapsing myelitis who developed extensive brain involvement followed by death.

Design: Case report.

Setting: Foothills Medical Center, Calgary, Alberta, Canada.

Patient: A 51-year-old woman with neuromyelitis optica spectrum disorder.

Results: Neuropathological examination disclosed neuromyelitis optica lesions, even in areas that appeared normal radiologically and grossly. Immunostaining confirmed the massive disintegration of astrocytes in the acute and chronic lesions, indicating that astrocytes are targeted early in the disease process. Induction of the immune response was demonstrated by reverse-transcriptase polymerase chain reaction analysis of relevant immune response genes.

Conclusions: This article supports and supplements current concepts of astrocyte disintegration in neuromyelitis optica and of immune mechanisms in its pathogenesis.

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Neuromyelitis Optica (NMO) is a central nervous system inflammatory disorder identified by diagnostic criteria and a biomarker antibody against the water channel aquaporin 4 (AQP-4). Myelin, oligodendrocyte, axonal, and astrocytic injury is felt to be mediated by antibody-dependent complement lysis as well as excitotoxicity from the combined loss of sodium-dependent glutamate transport and the excitatory amino acid transporter 2 that forms a complex with AQP-4 in the astrocyte membrane. The pathology is distinguished from antibody-mediated type II immunopathological change in multiple sclerosis by the differential distribution of immune complexes and the loss of detectable AQP-4 IgG staining in NMO lesions.

A 51-year-old woman presented in 2005 with a multilevel myelopathy (cervical level 2 to 5 and thoracic 4 to 7) 1 week following a herpes zoster–like rash in the C4 dermatome. She denied any episodes of optic neuritis. Cerebrospinal fluid testing revealed 94 leukocytes (90% lymphocytes), normal results of cytological examination, and no detectable varicella zoster DNA on polymerase chain reaction testing or varicella zoster virus antibodies. She had elevated antinuclear antigen antibodies and anti–Sjogren syndrome A antibodies without recurrent optic neuritis, and either of these conditions with cerebral lesions. Postmortem studies of NMO with extensive involvement of the brain, particularly the subcortical white matter, have only infrequently been published. Here we describe a case in which the active cerebral involvement of the terminal course and the innumerable subcortical lesions found after death enabled a detailed comparison of the early, intermediate, and more advanced lesions.
Clinical features. Results of testing of serum AQP-4 antibodies taken before treatment were negative. She received intravenous steroids, acyclovir, and neurorehabilitation, with significant residual deficit.

In 2008, she developed multifocal myelitis in the cervical and thoracic spinal cord. Results of testing for AQP-4 antibodies were positive, and she was treated with plasma exchange with initial stabilization. Within 3 months she manifested with progressive cognitive decline with the development of extensive cerebral white matter lesions and spinal cord involvement extending from the C2 to T1 levels (Figure 1). She was admitted to the intensive care unit with respiratory failure, despite receiving intravenous steroids and plasma exchange. She progressed to a vegetative state, with the development of pneumonia and ensuing death.

**NEUROPATHOLOGICAL FINDINGS**

Postmortem examination showed demyelinating and partially necrotizing lesions at all levels of the spinal cord, preferentially affecting posterior columns (Figure 2). The central gray matter was also involved, with relative sparing of anterior horn neurons. Active demyelinating lesions with moderate axonal destruction were seen in the optic nerves bilaterally. In addition, multifocal active demyelinating and partially necrotizing lesions were noted in the brainstem.

While there were grossly apparent lesions in the subcortical white matter and corpus callosum, far more numerous lesions were apparent only on microscopic examination. We identified 3 histological patterns and interpreted these to represent a sequence of early, intermediate, and more advanced lesions.

The early lesions (Figure 2, A and B) were characterized by relatively small size, perivascular location, and perivascular inflammation that included numerous eosinophils and few lymphocytes. Most of the lesions were perivenular but we found at least 1 centered on a small arterial vessel. Axonal transport, based on immunostaining for amyloid precursor protein, was impaired within the early lesions, and there was reduced immunostaining for glial fibrillary acidic protein (GFAP) and AQP-4. Demyelination in these early lesions was variable but relatively limited, and lesions could be found with loss of AQP-4 immunostaining and eosinophils but no convinc-
positive globules, and frequent GFAP-negative macrophages are shown bizarre cellular profiles (disintegrating astrocytes), innumerable GFAP immunostained for GFAP. Deep within the lesion, GFAP-positive enlarged seen (original magnification

Lesions in subcortical white matter. A, A very early lesion was stained with Luxol fast blue (LFB). Limited (if any) demyelination was seen within the lesion, which is surrounded by intact white matter. The modest perivascular inflammation included a few eosinophils (original magnification ×100). B, An early lesion was stained with hematoxylin-eosin. Early demyelination and microglial activation is seen, as well as modest perivascular inflammation including relatively frequent eosinophils and a few lymphocytes (original magnification ×400). C, An intermediate lesion was immunostained for glial fibrillary acidic protein (GFAP), including part of the perimeter of the lesion, which represents the confluence of smaller lesions, and encompasses several small vessels. There is diffuse loss of the normal background GFAP staining but also reactive astrocytes, not only at the perimeter of the lesion, but also within it (original magnification ×40). D, An intermediate lesion was immunostained for aquaporin 4, including part of the perimeter of the lesion, and showed loss of the normal aquaporin 4 immunopositivity within the lesion (right half of the figure) (original magnification ×40). E, An intermediate lesion was stained with LFB. Deep within the lesion, active but partial demyelination, occasional focal vesiculation of a myelin sheath, microglial activation, macrophages, and a few perivascular eosinophils are seen (original magnification ×400). F, An advanced lesion was stained for LFB, including part of the perimeter of the lesion, showing essentially complete demyelination within the lesion (upper left of the figure) and abundant macrophages (original magnification ×200). G, An advanced lesion was stained with Bielschowsky stain. Deep within the lesion, with abundant macrophages but relatively well-preserved axons are seen (original magnification ×200). H, An advanced lesion was immunostained for GFAP. Deep within the lesion, GFAP-positive enlarged bizarre cellular profiles (disintegrating astrocytes), innumerable GFAP-positive globules, and frequent GFAP-negative macrophages are shown (original magnification ×400).

The intermediate lesions (Figure 2, C-E) were larger, confluent, and histologically more advanced than the early lesions. Eosinophils were still evident around some of the vessels within these lesions, but in other parts of the lesion, perivascular eosinophils were less frequent or absent. Activated microglia characterized as small, irregular, and having CD68⁺ profiles were distributed diffusely in various parts of the lesion. In other areas, the same lesion sometimes contained more rounded CD68⁺ profiles and foamy macrophages accumulating around vessels. Immunostaining for GFAP revealed that, although these intermediate lesions still had a generalized (background) loss of the diffuse light GFAP staining of normal white matter, they also contained irregularly distributed reactive and degenerating astrocytes within the lesion (Figure 2C). Focally we found numerous round GFAP-positive profiles about 5 μm in diameter, suggesting swelled processes of the reactive and degenerating astrocytes. Significant but incomplete demyelination and impairment of axonal transport were diffusely present in the lesion as well as focally vesiculated myelin sheaths.

In the advanced lesion (Figure 2, F-H) we found no perivascular eosinophils but there were abundant macrophages localized around blood vessels. Demyelination was essentially total, with focal myelin sheath vesiculation particularly evident at the perimeter of the lesion; axons were relatively well preserved (Figure 2, F and G). The largest lesion of this type that we examined, located in close proximity to the ventricle, had innumerable GFAP-positive globules and occasional swollen bizarre GFAP-positive cellular profiles in the lesion, consistent with astrocytes in the final stage of disintegration (Figure 2H). Macrophages in these advanced lesions were largely GFAP-negative and most, if not all, of the GFAP-positive debris appeared to be extracellular.

**MOLECULAR STUDIES**

Semiquantitative real-time reverse transcriptase–polymerase chain reaction–based analysis was performed using previously described protocols and oligonucleotide primers.⁵ ⁷ It revealed that, relative to white matter from control patients who died of sepsis (n = 2), leukemia (n = 1), and Alzheimer disease (n = 2), HLA-DR and GFAP transcript abundance was increased in the spinal cord, optic nerve, cerebral white matter, and frontal cortex of the present case, although this effect was most evident for GFAP in the spinal cord and optic nerve (Figure 3). Additionally, CD3ε and CD8B transcripts were also increased in all regions of the central nervous system relative to the controls, especially in the spinal cord, albeit at comparatively lower levels than GFAP. Given that the tissues from the current case exhibited evidence of neuroinflammation, we also examined the relative expression of genes involved in tissue injury and chemotaxis and demonstrated induction of IL-6 and CXCL-10 relative to control white matter, particularly in the optic nerve. Surprisingly, AQP4 transcript levels did not differ in any anatomical site relative to the control white matter (data not shown).
Clinical features in this case were noteworthy, including an antecedent herpes zoster infection, a lack of clinically apparent visual impairment, and the terminally fulminant cerebral involvement by the disease. Relatively few case reports have described extensive brain or brainstem changes in patients with NMO.\textsuperscript{8-11} The radiological and pathological abnormalities in our case were supported by molecular analyses demonstrating induction of transcripts for genes mediating inflammation, particularly in the spinal cord and optic nerve.

The numerous active cerebral lesions in our case, and the comparatively large volume within which they were distributed, provided an opportunity to define the likely evolution from the smallest and presumably early lesions to the advanced lesions. Our findings are in accord with the interpretation that early lesions are perivascular and usually perivenular and include loss of both AQP-4 and GFAP immunostaining, influx of eosinophils and a few lymphocytes, impairment of axonal transport, early myelin breakdown, and early macrophage (microglial) activation. Around the perimeter, astrocytes are activated but also begin to disintegrate. As these early lesions expand, they become confluent and apparently overtake the peripheral reactive astrocytic process. The reactive astroglial network becomes condensed as it disintegrates. The normal diffuse background GFAP positivity is lost, while astrocytic perikarya and cell branches become swollen. Eosinophils eventually disappear. The most advanced cerebral lesion in our case was essentially totally demyelinated but had extensive axonal preservation. Unlike the classic NMO lesions in spinal cord, the cerebral lesions in our case showed little or no necrosis, despite the remarkable disintegration of astrocytes. We did not identify GFAP positivity within macrophages, suggesting a relative or absolute failure of the phagocytic cells to ingest the astrocytic debris.

Our anatomic findings support the concept that NMO targets astrocytes early in the pathogenetic sequence of events; yet, we found increased levels of GFAP messenger RNA and no change in the levels of AQP-4 messenger RNA. The tissue in which these transcripts were assessed was not microdissected. It included lesions as well as tissue surrounding the lesions, where astrocytes are reactive and likely upregulating various messenger RNA strands. The transcript levels we obtained are assumed to represent the aggregate of results from lesions and from perilesional reactive tissue. This probably accounts for the increased GFAP transcript levels as well as the normal AQP-4 transcript levels overall.

Our anatomic findings provide a coherent structural correlate for the previously proposed pathogenetic se-
quency and clinical findings. However, the cerebral lesions we examined in detail lacked the tissue necrosis that has been considered part of the classic NMO lesion. Clinically, the cerebral lesions were identified only late in the course of the disease, and they may represent a later stage of the disease progression than the spinal cord lesions. They occurred in the volumetrically larger setting of subcortical white matter. It is not clear whether the temporal and spatial differences from the spinal cord lesions account for the lack of necrosis in the cerebral lesions of our case. Nonetheless, the similarity of brain lesions account for the lack of necrosis in the cerebral lesions of NMO has been considered part of the classic NMO lesion. The similarity of brain and opticospinal lesions in NMO has been emphasized, and our findings are likely relevant to the earliest stages of disease progression. Passive transfer of NMO IgG from human patients can induce lesions in rats similar to those of NMO, including astrocytic damage, but tissue necrosis is not reported in that model.

In summary, we describe a patient with NMO who displayed extensive disease involvement at all levels of the neural axis. Astrocytes are identified as an important target of the disease, especially in the early phases, with evidence of immune activation. Our findings corroborate and extend current concepts of NMO pathology and pathogenesis.

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Financial Disclosure: Dr Lucchinetti reports having potential financial interest associated with technology related to this research. A patent has been issued for this technology (NMO-IgG testing), and it has been licensed to a commercial entity to develop a kit assay that will be available worldwide (not exclusive to Mayo Clinic). Dr Lucchinetti and Mayo Clinic have received royalties of less than the federal threshold for significant financial interest from the licensing of this technology and all have rights to receive future royalties.

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