doi:10.1093/brain/awq133 Brain 2010: 133; 1575–1577 | 1575

BRAIN
A JOURNAL OF NEUROLOGY

SCIENTIFIC COMMENTARY

Retinal pathology in multiple sclerosis: insight into the mechanisms of neuronal pathology

Although multiple sclerosis is commonly designated as an inflammatory demyelinating disorder of the central nervous system, historical and modern descriptions have underscored the involvement of axons and neuron cell bodies as germane to a more complete understanding of the pathophysiology of the disease (Raine and Cross, 1989; Ferguson *et al.*, 1997; Trapp *et al.*, 1998; Bitsch *et al.*, 2000; Peterson *et al.*, 2001; Lassmann, 2004; Bruck, 2005; Hauser and Oksenberg, 2006). Indeed, recent research has yielded evidence confirming neuronal apoptosis in multiple sclerosis plaque lesions, and signs of oxidative stress in cortex, hippocampus and deep grey matter structures (Peterson *et al.*, 2001; Bo *et al.*, 2003; Dutta *et al.*, 2006). Several studies have suggested a correspondence between the magnitude of the disease burden within the cerebral cortex and the predilection for having a progressive phenotype of multiple sclerosis (Kutzelnigg *et al.*, 2005; Lassmann *et al.*, 2007). The recently established paucity of inflammatory cell infiltrates and glial cell activation in grey matter lesions suggests that the pathobiological underpinnings of tissue injury in grey matter may be distinctive from that of white matter. In particular, grey matter injury could principally be the derivative of a primary neuronal mechanism of pathology, or perhaps secondary to indirect influences such as axonal injury and transection, culminating in neuronal degeneration and humoral injury cascades including antibodies, cytokines, proteases, nitric oxide and glutamate (Rudick and Trapp, 2009; Steinman, 2009). That multiple sclerosis neuronal pathology concomitantly exists in the eye and brain is also not a new principle, but rather has escaped extensive pathological re-exploration in the past few decades (Sharpe and Sanders, 1975; Fisher *et al.*, 2006; Gordon-Lipkin *et al.*, 2007; Sepulcre *et al.*, 2007; Waxman and Black, 2007). The occurrence of inflammation in the eye, as manifested by retinal periphlebitis and uveitis in patients with multiple sclerosis, has been recognized for many decades, but was only recently related to nerve fibre layer injury and brain atrophy. It is of further interest that, although retinal axons are unmyelinated (with myelin as the putative target of the disease process in multiple sclerosis), inflammatory activity within the eyes of patients with multiple sclerosis corroborates the hypothesis that the immune response in this disorder may be directed against antigens other than myelin, and establishes a precedent to explore actively innovative approaches that will characterize more fully these novel immune response repertoires. There has been a recent resurgence of interest in the anterior visual pathway as a window on the brain. The increased application of high-resolution optical coherence tomography has revealed that retinal nerve fibre layer thinning is a common occurrence in multiple sclerosis and exists independent of a history of optic neuritis (Trip *et al.*, 2005; Fisher *et al.*, 2007; Gordon-Lipkin *et al.*, 2007; Pulicken *et al.*, 2007; Sepulcre *et al.*, 2007; Henderson *et al.*, 2008). Bolstering the contention that occult disease activity targets the eye in a fashion similar to tissue injury in the brain and spinal cord, patients with primary progressive multiple sclerosis (those without inflammatory demyelinating attacks of any variety) also exhibit abnormally thinned retinal nerve fibre layers; and longitudinal changes in retinal nerve fibre layer thickness can be measured over time both in patients with and without a history of optic neuritis (Pulicken *et al.*, 2007).

In this issue of *Brain*, Green and colleagues report a post-mortem analysis of eyes from 82 cases of multiple sclerosis and 10 control patients with neurological disease. They report extensive retinal atrophy with shrunken neurons and dropout of both retinal ganglion cells in 79% of eyes, and inner nuclear layer (amacrine and bipolar cells) atrophy in 40% of eyes from people with multiple sclerosis. A subset of patients was examined by immunohistochemistry, which revealed human leucocyte antigen-DR reactive cells with a phenotype of microglia, and evidence of axonal loss and injury. There was also extensive glial fibrillary acid protein immunoreactivity consistent with astroglial cell activation. The severity of retinal atrophy was significantly associated with post-mortem brain weight and there was a trend towards an association with disease duration, suggesting that the observed pathology may be indicative of more global changes occurring in the brain over time. The conspicuous observation of frequent involvement of the iris in multiple sclerosis was also observed and associated with severity of the retinal pathology. None of these pathologic processes were observed in the control cases.

Limitations of this report include the categorical analysis of tissue pathology and skewing of samples for multiple sclerosis cases, with no central nervous system disease controls, and the limited number of cases examined for immunohistochemical staining (five cases providing a total of eight eyes). The absence of

detailed clinical histories and the less than optimal tissue preservation limited further clinicopathological correlation and ultrastructural analysis.

Nonetheless, this is the first description of inner nuclear layer cell loss in multiple sclerosis and is a reminder of the important lessons that can be learned from careful neuropathological examination. This report potentially represents a paradigm shift in our thinking about the mechanisms of neuronal disease, much in the way that the application of confocal microscopy strikingly revealed pervasive axonal transections and terminal ovoid profiles that rekindled interest in the relevance of axonal pathology in multiple sclerosis. While retinal ganglion cell dropout can be reconciled by understanding the relationship between optic nerve inflammation and consequent damage to nerve axons, the involvement of the inner nuclear layer is substantially more provocative. Specifically, the paucity of inflammatory mononuclear cells in the inner nuclear layer argues against a direct cell-mediated neuronal cytotoxic process. Green *et al*. postulate that this could instead reflect retrograde transsynaptic degeneration. Several recent studies have considered the relationship between transsynaptic degeneration and pathology within the anterior and posterior visual pathways (Audoin *et al.*, 2006; Reich *et al.*, 2009). Nevertheless, a histopathological signature for this process has yet to be demonstrated. The possibility that the inner nuclear layer pathology is related to humoral factors should be carefully interrogated, especially in light of the type I cortical (pial) pathology seen in multiple sclerosis, which may be mechanistically coupled to adjacent, but not necessarily contiguous, meningeal lymphoid follicles. Antibodies directed against neuronal elements, such as contactin 2, have also been described in multiple sclerosis (Derfuss *et al.,* 2009). Anti-retinal antibodies are recognized in cancers and, although less well characterized, there are analogous descriptions of anti-retinal antibodies associated with autoimmune disease (Heckenlively and Ferreyra, 2008; Adamus, 2009; Adamus *et al.,* 2009).

While not directly addressed in this study, the retinal epithelial layer is worthy of further analysis in multiple sclerosis. The retinal epithelial cells do not express major histocompatibility complex class II at rest but do so upon stimulation with interferon gamma. These cells are a reservoir for viral infections of the eye in humans and virus can propagate to other cells over time (Robbins *et al.*, 1990). Data from the intraocular infection of a corona virus, murine hepatitis virus (JHM strain), reveal that viral infection of the retinal epithelial cells can evoke markedly different retinal pathologies depending on the host's genetic background (Hooks *et al.*, 1993). In this model, BALB/c mice develop a retinal vasculitis followed by retinal degeneration, whereas CD-1 mice show retinal vasculitis but no retinal degeneration. Both strains develop early inflammation and antiviral antibodies, but the BALB/c mice have a late development of anti-retinal antibodies that is not seen in the CD-1 mice, suggesting a pathogenic role for these antibodies. Therefore, the possibility exists that a viral infection of the retinal epithelial cell layer could elicit a retinal degenerative process through retinal autoimmunity.

Optical coherence tomography could offer the ability to study retinal pathology over time *in vivo*. This technology could be used in order to increase our understanding of the temporal evolution of the pathology observed in this study and to interrogate the deeper layers including the retinal pigmented epithelial cell layer. Methods designed to segment the retinal nuclear topography that determines the underlying nerve fibre layer have been developed and could represent a powerful investigative tool by which we can begin objectively to quantify neuronal pathology in multiple sclerosis and other neurodegenerative disorders. The observation that nerve fibre layer thinning within the retina has been associated with reduced brain volumes suggests that intraocular pathologies may reflect more global pathology in the central nervous system. Indeed, in one study, nerve fibre layer was associated with cerebral white matter volume but not grey matter volume (Gordon-Lipkin *et al.,* 2007). Perhaps specific measurement of segmented nuclear cell layers will reflect cerebral cortical pathology better in specific subtypes of multiple sclerosis. A major caveat emphasized by the study of Green and colleagues is the presence of astroglial cells in the ganglion cell layer and microglial cells that extend down into the inner nuclear layer, which could confound measures of nuclear layer thickness.

Electroretinograms may also prove useful in examining for ganglion cell or inner nuclear layer pathology in multiple sclerosis. There are several old reports of abnormalities detected by electroretinography, but these were mostly in patients with optic neuritis and the abnormalities were attributed to retrograde degeneration (Persson and Wanger, 1984; Falsini *et al.,* 1999). Perhaps a more careful analysis could reveal cases in which there is evidence for primary retinal pathology.

Taken together, the findings by Green and colleagues of extensive retinal nuclear loss in both the ganglion and inner nuclear cell layers in eyes from patients with a history of multiple sclerosis provide clear documentation of neuronal pathology that in the case of the inner nuclear layer seems to be independent of classical mononuclear cell-mediated inflammation and myelin antigens. Resolving whether this is related to humoral mechanisms directly or indirectly targeting retinal neurons, or a manifestation of trans-synaptic degeneration, could provide major insights into the underpinnings of neuronal damage in multiple sclerosis.

Funding

National Multiple Sclerosis Society (PP1115 to L.J.B.); National Multiple Sclerosis Society Translational Research Partnership (TR 3760-A-3 to L.J.B., P.A.C.); National Institutes of Health/ National Eye Institute (K24 EY 014136 to L.J.B.); McNeill Foundation, and the DAD's Foundation.

Peter A. Calabresi,[1] *Laura J. Balcer*[2] *and Elliot M. Frohman*[3]
1 *Johns Hopkins University, Baltimore, MD, USA*
2 *University of Pennsylvania, Philadelphia, PA, USA*
3 *University of Texas Southwestern, Dallas, TX, USA*

Correspondence to: Peter A. Calabresi, MD,
Johns Hopkins Hospital, 600 N Wolfe St,
Pathology 627, Baltimore,
MD 212187, USA.
E-mail calabresi@jhmi.edu

References

Adamus G. Autoantibody targets and their cancer relationship in the pathogenicity of paraneoplastic retinopathy. Autoimmun Rev 2009; 8: 410–4.

Adamus G, Brown L, Weleber RG. Molecular biomarkers for autoimmune retinopathies: significance of anti-transducin-alpha autoantibodies. Exp Mol Pathol 2009; 87: 195–203.

Audoin B, Fernando KT, Swanton JK, Thompson AJ, Plant GT, Miller DH. Selective magnetization transfer ratio decrease in the visual cortex following optic neuritis. Brain 2006; 129(Pt 4): 1031–9.

Bitsch A, Schuchardt J, Bunkowski S, Kuhlmann T, Bruck W. Acute axonal injury in multiple sclerosis. Correlation with demyelination and inflammation. Brain 2000; 123(Pt 6): 1174–83.

Bo L, Vedeler CA, Nyland HI, Trapp BD, Mork SJ. Subpial demyelination in the cerebral cortex of multiple sclerosis patients. J Neuropathol Exp Neurol 2003; 62: 723–32.

Bruck W. The pathology of multiple sclerosis is the result of focal inflammatory demyelination with axonal damage. J Neurol 2005; 252(Suppl 5): v3–9.

Derfuss T, Parikh K, Velhin S, Braun M, Mathey E, Krumbholz M, et al. Contactin-2/TAG-1-directed autoimmunity is identified in multiple sclerosis patients and mediates gray matter pathology in animals. Proc Natl Acad Sci USA 2009; 106: 8302–7.

Dutta R, McDonough J, Yin X, Peterson J, Chang A, Torres T, et al. Mitochondrial dysfunction as a cause of axonal degeneration in multiple sclerosis patients. Ann Neurol 2006; 59: 478–89.

Falsini B, Porrello G, Porciatti V, Fadda A, Salgarello T, Piccardi M. The spatial tuning of steady state pattern electroretinogram in multiple sclerosis. Eur J Neurol 1999; 6: 151–62.

Ferguson B, Matyszak MK, Esiri MM, Perry VH. Axonal damage in acute multiple sclerosis lesions. Brain 1997; 120(Pt 3): 393–9.

Fisher E, Chang A, Fox RJ, Tkach JA, Svarovsky T, Nakamura K, et al. Imaging correlates of axonal swelling in chronic multiple sclerosis brains. Ann Neurol 2007; 62: 219–28.

Fisher JB, Jacobs DA, Markowitz CE, Galetta SL, Volpe NJ, Nano-Schiavi ML, et al. Relation of visual function to retinal nerve fiber layer thickness in multiple sclerosis. Ophthalmology 2006; 113: 324–32.

Gordon-Lipkin E, Chodkowski B, Reich DS, Smith SA, Pulicken M, Balcer LJ, et al. Retinal nerve fiber layer is associated with brain atrophy in multiple sclerosis. Neurology 2007; 69: 1603–9.

Hauser SL, Oksenberg JR. The neurobiology of multiple sclerosis: genes, inflammation, and neurodegeneration. Neuron 2006; 52: 61–76.

Heckenlively JR, Ferreyra HA. Autoimmune retinopathy: a review and summary. Semin Immunopathol 2008; 30: 127–34.

Henderson AP, Trip SA, Schlottmann PG, Altmann DR, Garway-Heath DF, Plant GT, et al. An investigation of the retinal nerve fibre layer in progressive multiple sclerosis using optical coherence tomography. Brain 2008; 131(Pt 1): 277–87.

Hooks JJ, Percopo C, Wang Y, Detrick B. Retina and retinal pigment epithelial cell autoantibodies are produced during murine coronavirus retinopathy. J Immunol 1993; 151: 3381–9.

Kutzelnigg A, Lucchinetti CF, Stadelmann C, Bruck W, Rauschka H, Bergmann M, et al. Cortical demyelination and diffuse white matter injury in multiple sclerosis. Brain 2005; 128(Pt 11): 2705–12.

Lassmann H. Recent neuropathological findings in MS–implications for diagnosis and therapy. J Neurol 2004; 251(Suppl 4): IV2–5.

Lassmann H, Bruck W, Lucchinetti CF. The immunopathology of multiple sclerosis: an overview. Brain Pathol 2007; 17: 210–8.

Persson HE, Wanger P. Pattern-reversal electroretinograms and visual evoked cortical potentials in multiple sclerosis. Br J Ophthalmol 1984; 68: 760–4.

Peterson JW, Bo L, Mork S, Chang A, Trapp BD. Transected neurites, apoptotic neurons, and reduced inflammation in cortical multiple sclerosis lesions. Ann Neurol 2001; 50: 389–400.

Pulicken M, Gordon-Lipkin E, Balcer LJ, Frohman E, Cutter G, Calabresi PA. Optical coherence tomography and disease subtype in multiple sclerosis. Neurology 2007; 69: 2085–92.

Raine CS, Cross AH. Axonal dystrophy as a consequence of long-term demyelination. Lab Invest 1989; 60: 714–25.

Reich DS, Smith SA, Gordon-Lipkin EM, Ozturk A, Caffo BS, Balcer LJ, et al. Damage to the optic radiation in multiple sclerosis is associated with retinal injury and visual disability. Arch Neurol 2009; 66: 998–1006.

Robbins SG, Hamel CP, Detrick B, Hooks JJ. Murine coronavirus induces an acute and long-lasting disease of the retina. Lab Invest 1990; 62: 417–26.

Rudick RA, Trapp BD. Gray-matter injury in multiple sclerosis. N Engl J Med 2009; 361: 1505–6.

Sepulcre J, Murie-Fernandez M, Salinas-Alaman A, Garcia-Layana A, Bejarano B, Villoslada P. Diagnostic accuracy of retinal abnormalities in predicting disease activity in MS. Neurology 2007; 68: 1488–94.

Sharpe JA, Sanders MD. Atrophy of myelinated nerve fibres in the retina in optic neuritis. Br J Ophthalmol 1975; 59: 229–32.

Steinman L. The gray aspects of white matter disease in multiple sclerosis. Proc Natl Acad Sci USA 2009; 106: 8083–4.

Trapp BD, Peterson J, Ransohoff RM, Rudick R, Mork S, Bo L. Axonal Transection in the Lesions of Multiple Sclerosis. N Engl J Med 1998; 338: 278–85.

Trip SA, Schlottmann PG, Jones SJ, Altmann DR, Garway-Heath DF, Thompson AJ, et al. Retinal nerve fiber layer axonal loss and visual dysfunction in optic neuritis. Ann Neurol 2005; 58: 383–91.

Waxman SG, Black JA. Retinal involvement in multiple sclerosis. Neurology 2007; 69: 1562–3.