NEWLY LESIONED TISSUE IN MULTIPLE SCLEROSIS—A ROLE FOR OXIDATIVE DAMAGE?

The causes of lesion formation in multiple sclerosis remain uncertain. While an autoimmune pathogenesis is favoured by many (Weiner, 2004; Frohman et al., 2006), innate immune mechanisms have also been proposed (Barnett and Prineas, 2004; Marik et al., 2007), in addition to roles for bacteria and viruses (Gay, 2007; Salvetti et al., 2009). This issue of *Brain* includes two papers on multiple sclerosis lesions, which may illuminate mechanisms involved in their formation (Haider et al., 2011) and repair (Zambonin et al., 2011).

Hans Lassmann and colleagues have explored their bank of multiple sclerosis tissue seeking information on oxidative damage (Haider et al., 2011). The authors uncover abundant evidence of oxidized lipids and DNA in active multiple sclerosis lesions, significantly adding to our understanding from earlier observations (Graumann et al., 2003; Gray et al., 2008b; Zeis et al., 2009; van Horssen et al., 2011). Lipid peroxides and oxidized nuclear DNA were mainly present in oligodendrocytes, often associated with evidence of apoptosis: a small number of reactive astrocytes were also affected. Oxidized phospholipids were also ‘massively’ accumulated in some axonal spheroids and neurons, many of which appeared to be degenerating.

Oxidative damage ensues when pro-oxidant factors (see below) overwhelm the inherent anti-oxidant defences of cells and tissues, resulting in oxidative stress and oxidative modification of biological molecules such as enzymes, lipids and DNA, thereby preventing normal cellular function and increasing the likelihood of cell death. The profound oxidative damage found in oligodendrocytes and axons is therefore important because it will undoubtedly contribute to the ongoing demyelination and axonal injury and degeneration, and perhaps may even be causative.

The existence of oxidative damage within active multiple sclerosis lesions is not in itself surprising, because the accompanying inflammation can be intense and there are several recognized sources of reactive oxygen species responsible for the protein, lipid and DNA oxidation. For example, it is well established that activated microglia are very effective producers of reactive oxygen species, not least by virtue of the NADPH oxidase-mediated respiratory burst, in which large amounts of superoxide can be produced extracellularly as a defence mechanism against invading microorganisms (Bedard and Krause, 2007). Activated microglia can also produce nitric oxide in large quantities through expression of the inducible form of nitric oxide synthase; and nitric oxide and superoxide can combine to produce the strong oxidizing agent peroxynitrite. Superoxide and nitric oxide are the lead agents in cascades of reactive oxygen and nitrogen species that can damage mitochondria, and importantly mitochondria—via multiple pathways (Brunori et al., 2006; Rubbo et al., 2009). The microglia can be activated in different ways, including by breakdown of the blood–brain barrier (which occurs in inflammatory multiple sclerosis lesions), and release from the vasculature of agents such as thrombin and fibrin that are found deposited on microglia in multiple sclerosis lesions (Marik et al., 2007). Alternatively, inflammation can also result in dysregulated glutamate homeostasis in multiple sclerosis lesions (Werner et al., 2001); and glutamate can activate receptors on oligodendrocytes and neurons causing the formation of reactive oxygen and nitrogen species by NADPH oxidase, and nitric oxide synthase (Karadottir and Attwell, 2007; Stys and Lipton, 2007; Brennan et al., 2009). It is, therefore, unsurprising that the oxidative damage found in the multiple sclerosis lesions is significantly correlated with ongoing inflammation, including the presence of microglia, macrophages and T cells (Haider et al., 2011).

The mechanisms by which oxidative damage can occur, impair mitochondrial function, and contribute to tissue damage in neuroinflammatory disease, have been summarized in a number of excellent reviews and papers (Andrews et al., 2005; Dutta et al., 2006; Dutta and Trapp, 2007; Kalman et al., 2007; Gonsette, 2008; Mahad et al., 2009; Witte et al., 2009; 2010; Mao and Reddy, 2010; Campbell and Mahad, 2011; Nakamura and Lipton, 2011; van Horssen et al., 2011). These studies describe the numerous ‘downstream’ consequences of oxidative and mitochondrial damage, including energy failure; but it is interesting to look at the new findings by Haider and colleagues (2011) and consider whether they might indicate earlier ‘upstream’ mechanism(s) contributing to the formation of newly lesioned tissue.

The immunoreactivity for oxidized lipids and DNA is not distributed uniformly across the lesions, as is clear from Fig. 1 (Haider et al., 2011). It is helpful to realize that the chronic active lesion
illustrated has grown over time (weeks or months). Although the lesion represents a continuum, it can helpfully be divided into stages and this is what Haider and colleagues have done. The centre of the lesion, termed the ‘late active’ region, is the oldest and it contains demyelinated axons. The centre is surrounded by the progressively younger regions of the ‘early active’ (active demyelination), then the ‘initial’ region (Gay et al., 1997; equivalent to the ‘prephagocytic’ region (Barnett and Prineas, 2004) with the pattern III pathology (Lucchinetti et al., 2000) of early demyelination), and these regions are surrounded by the ‘periplaque’ tissue, eventually merging with the normal appearing white matter (see Lassmann, 2011). Of these different regions, the periplaque has the least obvious pathology, but is perhaps the most interesting because it contains the advancing front suggesting that this contains the secrets of the earliest events responsible for converting normal-appearing tissue into multiple sclerosis lesions. Here, the new findings of Haider et al. (2011) regarding the distribution of the oxidative damage are surprising, and perhaps informative, because although it is greatest in the ‘initial’ region, where microglial activation is profound, the next most intense region is not towards the active region, which might have been expected, but rather outwards into the periplaque region. The new findings therefore imply significant oxidative stress in the periplaque; but what are its origins?

It seems that lymphocytes are absent from the parenchyma in the periplaque (Barnett and Prineas, 2004; Henderson et al., 2009), and present at only a low density (around one per vessel) in the perivascular spaces (Henderson et al., 2009). Thus, if judged simply by their density, T cells appear not to play a decisive role in orchestrating a pro-inflammatory environment (see also Barnett and Prineas, 2004; Mark et al., 2007; Henderson et al., 2009) unless their influence is propagated to the advancing front from where the T cells are abundant in the older portions of the lesion. However, expression of the neuronal and inducible forms of nitric oxide synthase are both upregulated in the periplaque (Liu et al., 2001; Zeis et al., 2009); and microglia are reported to be activated, with enlarged cell bodies and thickened ramified processes (Henderson et al., 2009). Data on the capacity for superoxide production, perhaps by the expression of functional NADPH oxidase, appear to be lacking in the multiple sclerosis literature, but the opportunity for oxidative damage is enhanced by the expression in some microglia/macrophages of myeloperoxidase (Gray et al., 2008a). If the microglia are appropriately equipped, the simplest explanation for the oxidative damage is therefore that the activated microglial cells (and perhaps astrocytes (Liu et al., 2001; Abramov et al., 2004)) are responsible for the oxidative damage, producing reactive oxygen and nitrogen species that affect oligodendrocytes and axons. Although some reactive species, including superoxide, are charged and their permeability across membranes is limited, others, including nitric oxide, pass membranes easily and so will gain access to the intracellular space of oligodendrocytes and axons.

It is worth pausing here to note that although it is easy to assume that the microglial cells become activated and then inflict oxidative damage on the oligodendrocytes and axons, it remains possible that the primary pathology is in the oligodendrocytes and that the microglia are reacting to it; or, especially, that both mechanisms are operating together in mutual reinforcement. Indeed, in another study the microglial cells surrounding newly forming lesions remained unactivated in two cases of acute multiple sclerosis (Henderson et al., 2009), consistent with the microglia having a more reactive, than proactive, role. It is therefore worthwhile considering whether the oligodendrocytes and axons might not be innocent targets of the oxidative damage and lesion formation, but whether they may be partly responsible. How might this occur?

Oligodendrocytes and axons contain many mitochondria and these can be an important source of reactive oxygen species. In fact mitochondria produce some superoxide under normal conditions as a consequence of ‘leakage’ of electrons onto oxygen as they move along the electron transport chain (Murphy, 2009a; Stowe and Camara, 2009); and although this can be important in physiological signalling (Murphy, 2009a), the production can increase substantially under various conditions with inherent antioxidant defences thereby overwhelmed, resulting in oxidative stress and damage (Andrews et al., 2005; Kalman et al., 2007; Murphy 2009a; Stowe and Camara, 2009; Witte et al., 2010; van Horsen et al., 2011). The slow rate of advance of multiple sclerosis lesions suggests that a status quo may initially be maintained during which the antioxidant defences are capable of restraining runaway oxidative damage (Zeis et al., 2008). Eventually however, either by slow attrition, or the superimposition of other adverse events (e.g. systemic infection), homeostasis is lost and the lesion advances as the oligodendrocytes (especially) and axons succumb.

Perhaps, the most important determinants of mitochondrial superoxide production are: (i) mitochondrial damage such that normal function is prevented; (ii) whether the mitochondria have a high proton motive force, namely a high membrane potential and pH gradient; (iii) when the pool of coenzyme Q is reduced (iv) a high nicotinamide adenine dinucleotide/NAD+ ratio; and (v) an unusually high or low oxygen concentration (Murphy, 2009a). Condition (i) is likely to occur because nitric oxide (and some other reactive oxygen and nitrogen species) from activated microglia will freely diffuse into the mitochondria of oligodendrocytes and axons and then either modify the mitochondrial constituents directly, or, in the case of nitric oxide, combine with mitochondrial superoxide to form the strong oxidizing agent peroxynitrite. This agent can permanently nitrate tyrosine residues (i.e. damage the associated proteins), and there is good evidence for mitochondrial damage and nitrotyrosine formation in multiple sclerosis lesions (Liu et al., 2001; Mahad et al., 2009; Zeis et al., 2009). As the mitochondria become damaged by the toxic environment their own production of reactive oxygen species can increase, setting up a vicious cycle.

Conditions (ii), (iii) and (iv) are all favoured when ATP production is low. This may occur in axons, if demand is reduced by conduction block—expected in inflamed, demyelinated axons, especially if the main body of the lesion is proximal to the direction of impulse conduction (Smith et al., 2006). The effects of inflammation on the mitochondrial membrane potential are difficult to predict, but observations in vivo in experimental autoimmune encephalomyelitis (EAE) favour membrane depolarization (Qi et al., 2006; Nikic et al., 2011), although membrane hyperpolarization has been documented due to nitric oxide in a different
preparation, operating through the reverse action of the ATPase supported by upregulation of glycolysis (Beltran et al., 2002). Which condition would prevail in the periplaque is not known, but it might be expected that ATP production would be reduced by inhibition of mitochondrial complex IV of the respiratory chain by nitric oxide. Such inhibition has long been suspected in multiple sclerosis lesions (Redford et al., 1997) because nitric oxide is a potent inhibitor of this enzyme (Bolanos and Almeida, 2006) and the nitric oxide concentration is expected to be high (Smith and Lassmann, 2002).

Although oxygen concentration is an important determinant of the mitochondrial production of reactive oxygen species [Murphy, 2009a: condition (v)], the concentration of oxygen within multiple sclerosis lesions is not known. Hyperoxia is a well-established cause of increased reactive oxygen species production and could occur if mitochondrial utilization of oxygen is reduced by nitric oxide-mediated inhibition of complex IV of the respiratory chain (Hagen et al., 2003). A hypoxic environment is however favoured by the very prominent expression of the hypoxia inducible factor $\alpha$ (HIF-1$\alpha$) within pattern III multiple sclerosis lesions (Aboul-Enein et al., 2003; although HIF-1$\alpha$ accumulation is not specific for hypoxia (Palmer et al., 2000; Bove et al., 2008; Majmundar et al., 2010; Olson and van der Vliet 2011)) and also by separate evidence for hypoxia in an experimental pattern III lesion in vivo (Desai et al., 2011). If the environment is hypoxic, it is not safe to assume that production of reactive oxygen species will be low (due to reduced availability of oxygen), as some evidence indicates that the mitochondrial release of reactive oxygen species increases in hypoxia, and in fact this appears to be necessary for the observed stabilization of HIF-1$\alpha$ to occur (Klimova and Chandel, 2008). There are thus several reasons to believe that the mitochondrial production of reactive oxygen species will increase in multiple sclerosis lesions.

Under the different conditions detailed above, the mitochondrial superoxide production originates primarily from complex I of the respiratory chain, and the superoxide is formed on the matrix side of the inner mitochondrial membrane, namely in the same compartment as the mitochondrial DNA. Mitochondrial DNA is particularly vulnerable to oxidative damage (Yakes and Van Houten, 1997), and indeed mitochondrial DNA defects are well documented in multiple sclerosis (Mao and Reddy; 2010; Campbell et al., 2011), reinforcing a view that the mitochondrial production of reactive oxygen species may increase in multiple sclerosis lesions.

If mitochondria are an important source of reactive oxygen species, as well as ATP, they can be viewed not only as helpful ‘saviours’ in a battle against impending energy deficits, but also as risky components that increase the chance of triggering an oxidative firestorm with microglia (Campbell and Mahad, 2011; van Horssen et al., 2011) even from a quiescent beginning. Especially with this in mind, the new findings in a second paper published in this issue of Brain, from Don Mahad and his colleagues, are very interesting. Zambonin et al. (2011) reported that established remyelinated axons in multiple sclerosis lesions contain more mitochondria than normal, but fewer mitochondria than established demyelinated axons. If axonal mitochondria are a potential source of excessive production of reactive oxygen species, the increased number of mitochondria in remyelinated (and demyelinated) axons (Campbell and Mahad, 2011) may place the axons at increased danger for degeneration; and this is consistent with findings in multiple sclerosis. Thus, some remyelinating and remyelinated axons in multiple sclerosis can have greater evidence of axonal injury than occurs in established demyelinating lesions (Kuhlmann et al., 2002).

In this commentary, it has been reasoned that it may be possible to look beyond the boundary of the chronic active multiple sclerosis lesion into the periplaque region, in order to explore the earliest events as an existing lesion grows, but it is interesting to wonder whether mechanisms responsible for the extension of existing lesions might recapitulate those involved with the formation of new lesions. This question is difficult to answer in multiple sclerosis tissue, so is there any evidence from experimental studies that oxidative damage might occur in the normal nervous system as an initial event in lesion formation? An unexpected finding in EAE is pronounced oxidative and nitrative injury of tissue and mitochondria as early as 3-days post-immunization, well before the onset of obvious inflammation marked by the infiltration of inflammatory cells (Qi et al., 2007a). This finding is intriguing, not least because similar findings are reported in animals with experimental autoimmune uveitis (Saraswathy and Rao, 2008). The source of the oxidative damage remains unclear, but innate immune mechanisms seem likely and this interpretation is supported by other findings in EAE, although using a very different model (Ponomarev et al., 2005). Although not conclusive, the experimental findings show that oxidative damage can be a very early event in the formation of new autoimmune lesions.

In summary, new observations regarding oxidative damage in chronic active multiple sclerosis lesions might reflect the early events occurring as the tissue is lesioned in multiple sclerosis. Thus, if the periplaque tissue can be interpreted as the advancing edge of the slowly growing lesion, it is interesting that it exhibits clear oxidative damage in the presence of activated microglial cells expected to be releasing superoxide and nitric oxide and the cascade of reactive oxygen and nitrogen species that follows. Several reasons are presented to suspect that reactive oxygen species produced by the glial and axonal mitochondria may add to the oxidative damage experienced by these cells, exacerbating the mitochondrial damage, including damage to the mitochondrial DNA. If so, anti-oxidant therapy could be beneficial in multiple sclerosis and there is indeed supporting evidence for this approach from experimental studies (Qi et al., 2007b; Nikic et al., 2011).

Such therapy may inhibit the growth of existing multiple sclerosis lesions, and perhaps prevent the formation of new ones. It may also prevent damage to demyelinated and remyelinated axons. As mitochondria are damaged in the disease process, and may also contribute to the oxidative stress, anti-oxidant therapies targeted directly at mitochondria (Murphy, 2009b) may be particularly effective.

Acknowledgements

I am grateful to Michael Duchen, Michael Murphy and Jia Newcombe for their expert comments on the manuscript.
Funding

Work in the author’s laboratory is supported by grants from the Brain Research Trust, the Medical Research Council and the Multiple Sclerosis Society of Great Britain and Northern Ireland.

Kenneth J. Smith
Department of Neuroinflammation, UCL Institute of Neurology,
Queen Square, London WC1N 3BG, UK

Correspondence to:
Kenneth J. Smith,
Department of Neuroinflammation,
UCL Institute of Neurology,
Queen Square,
London WC1N 3BG, UK
E-mail: k.smith@ion.ucl.ac.uk
Advance Access publication June 15, 2011
doi:10.1093/brain/awr144

Reference

Abramov AY, Canevari L, Duchen MR. Beta-amyloid peptides induce mitochondrial dysfunction and oxidative stress in astrocytes and death of neurons through activation of NADPH oxidase. J Neurosci 2004; 24: 565–75.


Yakes FM, Van Houten B. Mitochondrial DNA damage is more extensive and persists longer than nuclear DNA damage in human cells following oxidative stress. Proc Natl Acad Sci USA 1997; 94: 514–9.

