The role of nitric oxide in multiple sclerosis

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Nitric oxide (NO) is a free radical found at higher than normal concentrations within inflammatory multiple sclerosis (MS) lesions. These high concentrations are due to the appearance of the inducible form of nitric oxide synthase (iNOS) in cells such as macrophages and astrocytes. Indeed, the concentrations of markers of NO production (eg, nitrate and nitrite) are raised in the CSF, blood, and urine of patients with MS. Circumstantial evidence suggests that NO has a role in several features of the disease, including disruption of the blood–brain barrier, oligodendrocyte injury and demyelination, axonal degeneration, and that it contributes to the loss of function by impairment of axonal conduction. However, despite these considerations, the net effect of NO production in MS is not necessarily deleterious because it also has several beneficial immunomodulatory effects. These dual effects may help to explain why iNOS inhibition has not provided reliable and encouraging results in animal models of MS, but alternative approaches based on the inhibition of superoxide production, partial sodium-channel blockade, or the replacement of lost immunomodulatory function, may prove beneficial.

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Since the realisation in the late 1980s that nitric oxide (NO) has several important physiological roles in biological systems, there has been an increasing recognition of its importance in many diseases, including multiple sclerosis (MS). This review describes some of these roles; the consequences of NO production in MS are numerous and include both beneficial and deleterious effects. This dual action of NO helps to explain why, despite initial optimism, therapy for MS through the control of NO production has proven difficult to develop.

The chemistry of NO is complex,[1,2] even when studied in vitro, and is poorly understood in vivo, particularly in inflamed tissue. Such tissues contain many different microenvironments that may each affect the chemistry of NO in different ways (eg, they may differ in redox conditions, pH, or oxygen tension) and the cells involved may also have different capabilities, or defences, for handling nitrative and oxidative stress. Because NO is a reactive molecule, it does not exist in tissues only as a free radical; it also gives rise, sometimes reversibly, to several other related compounds. These compounds include the nitroxyl (NO^-) ion, nitrous acid (HNO_2), the nitrogen dioxide (NO_2) radical, peroxynitrite ($ONOO^-$; a product of the combination of superoxide and nitric oxide) and peroxynitrous acid (ONOOH). Several of these molecules are also reactive and have their own chemistries and biological activities.[2–5] The forms that NO takes at sites of inflammation are not known with much certainty, and so in this review the term "NO" is used to include not only the free radical but also its related compounds, often referred to collectively as reactive nitrogen species (or reactive nitrogen intermediates). The above complexities are increased further by the fact that NO is a potent signalling molecule, so its presence can change the behaviour of cells, including that of the cells that directly create or control pathological processes. All these complications mean that understanding of the role of NO in MS will remain a challenge for many years to come, but the challenge must be met because NO has several major and direct roles in the disease process. For example, NO seems to be involved in the immunological processes leading to lesion development, the processes leading to the limitation of lesion development, and in the mechanisms underlying the production of neurological deficit.

Evidence that NO concentrations are raised in MS

There is abundant evidence that the production of NO is significantly raised within MS lesions, arising not only from the pathological study of lesions themselves, but also from studies of the CSF, blood, and urine of patients, and the electron paramagnetic resonance spectroscopy of animals with experimental autoimmune encephalomyelitis,[6] a recognised model for MS.

Experimental autoimmune encephalomyelitis and MS lesions

The normal CNS contains the two main constitutively expressed forms of nitric oxide synthase (NOS), endothelial and neuronal NOS (eNOS and nNOS).[7] These forms of the enzyme produce low (ie, nanomolar) concentrations of NO in a calcium-dependent manner, and in this way NO contributes to the regulation of blood flow and participates in some synaptic transmission. However, the production of NO is much higher at sites of inflammation owing to the appearance of the inducible form of the enzyme, iNOS

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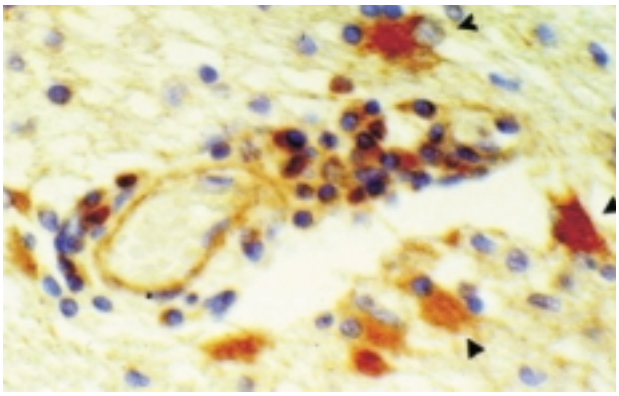

Figure 1. iNOS immunoreactivity (brown) in an acute MS lesion from a patient with Balo's concentric sclerosis. Reproduced with the permission of the American Society for Investigative Pathology.[16]

(the "i" stands variously for inducible, immunological, inflammatory, or independent of calcium). This form of the enzyme does not depend on free calcium, and it produces NO continuously, which results in much higher NO concentrations. iNOS is not normally present within the CNS, but iNOS mRNA[8] and protein[9,10] become expressed within the inflammatory lesions of animals with experimental autoimmune encephalomyelitis, and the expression coincides temporally and quantitatively with the severity of clinical signs.[8,11] In MS, iNOS mRNA was abundantly expressed in a biopsy sample taken within 33 days of the onset of disseminated symptoms in a patient with Marburg's-type MS[12] (this finding indicates that NO production is likely to be present from early in the disease process). iNOS mRNA was also found in the brains of all seven MS patients examined in another study[13] (see also 14). iNOS protein has also been found immunohistochemically in acute lesions (figure 1),[15,16] but as lesions become less inflammatory, as in chronic active disease, iNOS labelling diminishes.[16,17] The established demyelinated plaques of chronic MS do not express iNOS.[15] iNOS labelling occurs in cells of the microglial–macrophage lineage,[15,17,18] and reactive astrocytes.[16] iNOS-positive endothelial cells have also been described.[16] Formation of the strong oxidising agent peroxynitrite within MS lesions is indicated by the presence of intense nitrotyrosine labelling in most active MS lesions,[16] with labelling of hypertrophic astrocytes,[15,19] and, especially, iNOS-positive macrophages/microglial cells.[13,15,18,19]

Analysis of CSF

Much of the NO produced within the body is ultimately converted to nitrite and nitrate, so several studies have examined the concentrations of these ions in the CSF, blood, and urine of patients with MS. Although two studies involving only small numbers of patients found that the concentrations in the CSF were no higher than in healthy people,[20,21] other studies have found significantly raised nitrite and nitrate concentrations.[19,22–27] So far, the correlation between clinical disease activity and nitrite and nitrate concentrations in the CSF is weak, which may be explained partly by subclinical disease activity in clinically stable patients. As might be expected, CSF nitrite and nitrate concentrations were significantly lower when measured 1–2 months after treatment with methylprednisolone.[26]

The CSF from patients with MS also contains substances (perhaps cytokines) that promote the production of NO by glial cells in vitro.[28]

Serum analysis

The concentrations of nitrite and nitrate in the serum of patients with MS have been described as being lower than,[29] the same as (in primary progressive MS),[25] or higher than[23,30] those in controls. This rather unsatisfying summary may arise from the fact that increases in nitrite and nitrate concentrations that may be present within the CNS will be greatly diluted in the serum. Furthermore, serum concentrations will be disturbed in patients and healthy people by peripheral sources of nitrite and nitrate, including those from any peripheral inflammation and those arising from the diet. Zabaleta and colleagues[31] found that the concentration of nitrotyrosine (a stable marker of peroxynitrite production) was about six times higher in the serum of patients with MS than in controls ($p<0{\cdot}0001$); the patients with the highest values were those with chronic progressive disease. In rats with experimental autoimmune encephalomyelitis, the concentration of reactive nitrogen intermediates in the serum was found to be raised during the recovery phase.[32]

A particularly interesting finding is that the formation of NO in patients with MS can cause the production of neoantigens on proteins by the S-nitrosation of cysteine. The antibodies formed against such epitopes have been found in significantly raised titres in patients with all clinical forms of MS.[33]

Urine

Urine provides a readily available fluid suitable for serial sampling, but it has been little studied in MS. It is therefore noteworthy that nitrite and nitrate concentrations in urine were significantly higher in patients with demyelinating disease (most of whom had the relapsing–remitting or progressive forms of MS) than in healthy controls; however, the concentrations were not related to clinical relapses or magnetic resonance imaging evidence of lesion activity.[34]

Role of NO in inflammation and demyelination in MS

NO may be involved in the development of several pathological features of MS, the hallmark of which is the demyelinated plaque with reactive glial scar formation.[35] The demyelination occurs in association with a chronic inflammatory process, dominated by the infiltration of T lymphocytes, recruitment of haematogenous macrophages, and the local activation of microglia.[36] The inflammatory process is associated with a disturbance of the blood–brain barrier (BBB) that is most extensive in active lesions[37,38] but also persists at a more limited level in chronic plaques.[39] The essential structural damage within the plaques is primary demyelination with relative axonal preservation. However, emphasis has to be laid on the term relative, because some axons are lost in all cases, although the degree

of loss varies between different lesions.[40] In addition, remyelination can occur in MS lesions, particularly at early stages of the disease.[41,42] NO may have roles in each of these processes because it can have direct effects on the permeability of the BBB, and the induction and control of the inflammatory process. NO might also have a role in the mediation of demyelination and oligodendrocyte destruction and the functional and structural injury of axons.

Direct effects of NO on cerebral vessels and the BBB

NO has two major effects on cerebral vessels, both of which may be involved in the pathogenesis of MS lesions—namely, vasodilation and a disturbance of the BBB. NO can arise in lesions from various sources, including nerve terminals,[43,44] the induction of iNOS,[45] or the release of neurotransmitters,[46,47] and it provokes profound vasodilatation of the cerebral vasculature in both normal and pathological conditions.[48] Vasodilation by itself may facilitate inflammation by decreasing the velocity of blood flow, thereby aiding the binding of leucocytes to the vasculature and their migration through it. In addition, in pathological conditions, NO-mediated vasodilation generally occurs in conjunction with a disturbance of the permeability of the BBB, which will promote the passage of inflammatory cells and mediators into the CNS parenchyma. Indeed, the integrity of the BBB is well known to be compromised in MS at the sites of inflammatory lesions, such that the focal leakage of intravascular contrast agents (eg, gadolinium-diethylenetriaminepenta acetic acid) on magnetic resonance imaging is regarded as a marker of inflammation.[49] In agreement with this belief, the nitrite and nitrate concentrations in CSF of patients with MS are correlated with BBB breakdown, as measured by the leakage of albumin.[23]

Although NO can disturb measures of BBB integrity in vitro[50] and NO donors can increase BBB permeability,[51,52] the precise molecular mechanisms mediating NO-induced breakdown of the barrier are complex and incompletely understood.[53] Different NOS can be involved,[54] and the interaction of different NO redox species may occur synergistically.[55] In some, but not all, models a role for cyclic guanosine monophosphate has been suggested,[50,56,57] and BBB damage is massively augmented by the simultaneous formation of reactive oxygen species, in particular the formation of peroxynitrite.[52,54,58]

In inflammatory conditions, iNOS appears to be the main producer of NO,[59] but eNOS also contributes, and may have counter-regulatory effects through the inhibition of TNF-α-induced NF-κB signalling and COX-2 transcription.[60] In this way, NO produced in inflammatory conditions may have both proinflammatory and anti-inflammatory actions at the BBB.

Beneficial and immunomodulatory effects of NO

NO has long been regarded as a major factor aggravating inflammation in the CNS, but recent studies aimed at treating experimental autoimmune encephalomyelitis by the administration of relatively specific inhibitors of NOS have provided confusing results. The findings indicate that NO may on the one hand exert a proinflammatory action, by disturbing, for example, function of the BBB and having cytotoxic properties, but may on the other hand help to control the immune response via several immunoregulatory processes.

T-cell-mediated brain inflammation is induced and controlled at several levels. Autoreactive T lymphocytes are activated through peripheral antigen challenge,[61] which induces antigen-dependent T-cell proliferation and activation in peripheral lymphatic tissues. Activated T cells can enter the CNS compartment in the process of immune surveillance and, when they encounter their specific antigen, they start the proinflammatory cascade by producing cytokines.[62,63] This cascade results in the production of chemokines in the CNS[64] and the expression of adhesion molecules[65] at the blood–brain interface. These molecules are then instrumental in the recruitment of additional antigen-specific T cells into the brain, but also in secondary infiltration of the tissue with other T cells and effector cells such as macrophages.[66] This proinflammatory cascade is effectively counter-regulated by the destruction of T cells within the CNS through apoptosis,[67,68] which involves not only the autoreactive T cells, but also those that are unspecifically recruited into the lesions.[69] This apoptotic destruction of T cells is apparently so effective that, at least in acute monophasic brain inflammation, the encephalitic process is maintained only while new T cells can be recruited into the lesions from the peripheral immune system.[70] NO can interfere with this inflammatory cascade at several steps, thus assuming an anti-inflammatory role. NO can inhibit antigen presentation[71] and T-cell proliferation,[72] and thus it may limit the recruitment and activation of autoreactive T lymphocytes. Furthermore, through a downregulation of adhesion-molecule expression[73] it may inhibit the recruitment not only of T cells but also of macrophages into the lesions. Within the experimental autoimmune encephalomyelitis model, the severity of clinical disease and immune-mediated tissue damage is determined by the macrophage infiltration into the lesions.[74]

NO may also affect the clearance of inflammation in the CNS. Induction of T-cell apoptosis depends at least partly on the presence of NO,[75] and encephalitogenic T lymphocytes are highly susceptible to apoptosis induced by reactive NO species.[76] Within experimental autoimmune encephalomyelitis lesions, apoptotic T cells colocalise with iNOS-reactive macrophages and, in part, label for nitrotyrosine. Thus, some of the immunomodulatory activities of NO and its related compounds may be intricately linked to their toxic actions, in this case directed against the population of pathogenetic autoreactive T cells. This action may occur in both the peripheral immune system and within the lesion itself, although in a detailed study of T-cell apoptosis in experimental autoimmune encephalomyelitis lesions in iNOS-deficient animals, no significant reduction of T-cell apoptosis was observed.[77]

In the light of all these immunomodulatory effects, it is less surprising that the consequences of NOS inhibition in experimental autoimmune encephalomyelitis are sometimes adverse.

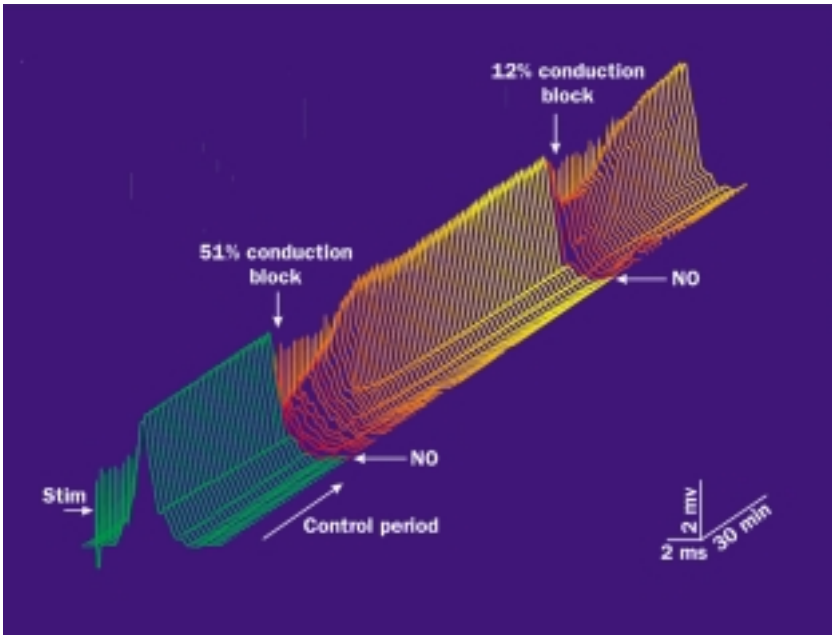

Figure 2. A series of compound action potentials from a pathway that includes a central demyelinating lesion. The records were obtained 2 min apart, and the plot shows about 5 h of recording time. For the first 1 h (green) the records were stable, but the injection of an NO donor into the lesion caused a prompt reduction in the amplitude of the compound action potential, consistent with conduction block in about 50% of the axons. The conduction block slowly reversed as the donor was depleted but was reimposed in fewer axons by a later, similar injection of a lower donor concentration. Reproduced with the permission of Oxford University Press.[95]

Oligodendrocyte injury

Primary demyelination is the most characteristic feature of MS lesions. The process of demyelination is usually associated with injury to, and loss of, oligodendrocytes, although the magnitude of this injury varies substantially between patients.[78] The mechanisms that cause oligodendrocyte injury are not clearly understood and may vary between patients.[79] A potential role for NO is implicated by observations in vitro that oligodendrocytes are much more sensitive to NO-mediated toxicity than astrocytes and microglia,[80] despite experiencing similar degrees of mitochondrial impairment and DNA damage.[80] NO can induce cell death through necrosis[81–83] or apoptosis[82,84] depending, at least partly, on the NO concentration, duration of exposure,[82,83] and the stage of cell differentiation.[85] Some of the effects of NO on oligodendrocytes (and other cells) may be mediated by promotion of DNA strand breaks, with the subsequent energy-consuming action of poly(ADP-ribose) synthase.[86] Peroxynitrite or other NO-related species might also damage oligodendrocytes by activating matrix metalloproteinases (some of which can degrade myelin constituents) from their proenzyme forms.[87]

Another way in which reactive nitrogen species, especially peroxynitrite, might damage oligodendrocytes and myelin is by lipid peroxidation,[88] which can affect not only membrane fluidity and permeability, but also the properties of membrane proteins. The type of lipid modification effected by peroxynitrite can change it into a form that is recognised by the macrophage scavenger receptor,[89] which may be relevant to demyelination. Also, because the phagocytosis of CNS myelin can stimulate macrophages to produce substantial amounts of NO,[90] there is the theoretical possibility of a positive feedback reaction.

A recent publication has suggested that NO might exert a moderately protective function in the cuprizone model of central demyelination.[91] However, in this model the demyelination is believed to be due to a direct toxic effect of cuprizone on oligodendrocytes, so the role of inflammation, and of NO, in most of the demyelination is difficult to ascertain.

In summary, on the basis of largely in vitro observations, NO may be important in demyelination, but it is most unlikely to be the only cause of myelin destruction in inflammatory brain lesions.

NO and loss of function in MS

It seems likely that NO has a role in the loss of neurological function in MS, perhaps even a major role, although the evidence is so far only circumstantial.

NO and conduction block

Demyelination has long been known to impair conduction,[92] and the loss of function in MS was, for many years, primarily attributed to the associated and prominent demyelination. However, several observations have now emphasised that inflammation may also be important;[49,93] a recent biopsy study (in which iNOS was abundant in the inflammatory lesions studied)[12] concluded that "inflammation alone may be sufficient to cause significant clinical deficits without demyelination".[94] The initial suspicion that the cytokines might directly cause impairment of axonal conduction was not supported by experiments,[95] but cytokines can be expected to promote the appearance of iNOS within lesions (because a residual inflammatory response can persist at such sites), resulting in NO production around damaged axons. Such production might be expected to cause clinical exacerbations because NO can promptly and reversibly block axonal conduction[83,95,96] and demyelinated axons are particularly vulnerable to this effect (figure 2).[95] Thus at low NO concentrations demyelinated axons would be affected selectively; this finding provides a plausible explanation for the transient exacerbation of earlier symptoms observed on administration of humanised monoclonal antibody to CD52.[93]

The mechanisms by which NO might cause conduction block are not yet clear, partly because NO has several effects on axons that could each have a role. Perhaps the simplest explanation is that NO can directly impair the function of sodium channels,[97–99] including blocking them,[100,101] and some of these effects are apparent with endogenous NO

production.[98,100,102] Mechanisms involving the nitrosation of critical channel thiols[97,98,103] and the action of cyclic guanosine monophosphate[99] seem to be involved. However, NO or nitrosothiols[2] also affect other axonal channels, including potassium and calcium channels,[104] and these effects may also impair conduction. Although calcium channels are not prominently expressed along the normally myelinated portions of axons, they can appear at sites of demyelination,[105] and if functionally expressed in the membrane they would be expected to affect conduction. NO might also impair conduction by affecting calcium channels in axon terminals. NO could also block conduction by depolarisation of axons,[83] either by an effect mediated by cyclic guanosine monophosphate,[106] or perhaps by direct effects on the electrogenic sodium–potassium ATPase (sodium pump).[107] Depolarisation could also result from the well-established ability of NO to impair mitochondrial metabolism, which would result in inadequate ATP production to maintain the electrogenic pumps.

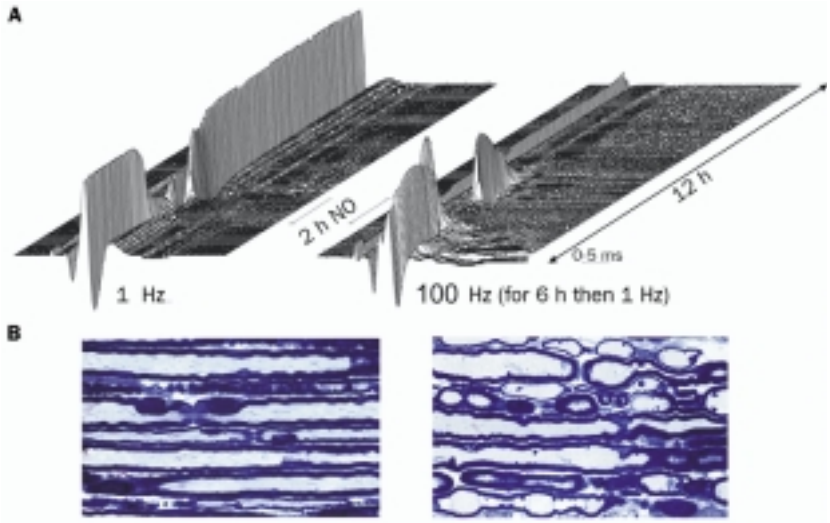

Figure 3. Two series of compound action potentials recorded in parallel from two dorsal roots (A). The root illustrated on the left was stimulated at 1 Hz throughout, whereas the other root (right) was stimulated at 100 Hz for the first 6 h, and at 1 Hz thereafter. Records were obtained from each root every 2 min over 12 h total recording time and the roots were exposed to NO for 2 h during the period indicated. NO exposure caused conduction block in both roots, but whereas the block was reversible in the root stimulated at 1 Hz, the combination of NO exposure and impulse activity (right) resulted in only a partial and temporary recovery, and this preceded a persistent period of conduction failure. On histological examination of the roots at the end of the experiment (B) the left root appeared normal, but the experience of electrical activity and NO exposure resulted in axonal degeneration (right). Reproduced with the permission of J Wiley and Sons inc.[124]

NO and synaptic transmission

Raised concentrations of NO also have adverse effects on synaptic transmission that, in addition to compromising transmission in motor and sensory pathways, may help to explain the loss of cognitive function in patients with MS.[108] NO has effects on synapses as part of normal physiological function,[109] but in MS much higher, pathophysiological concentrations can be expected near synapses because inflammatory, iNOS-expressing lesions can be common within the grey matter.[110,111] The high NO concentrations may well simply swamp the normal, delicately balanced, processes of synaptic function. However, NO in each of several different forms can directly affect NMDA and α-amino-3-hydroxy-5-methyl-4-isoxasole propionic acid (AMPA) receptors.[112] Indeed, a vicious cycle has been hypothesised, involving sodium channels, glutamate release, NMDA receptors, and local NO production, that acts synergistically to cause degeneration.[113] The finding that antagonists of AMPA receptor function provide protection in the animal model of experimental autoimmune encephalomyelitis may indicate an important role for these receptors in MS.[114,115]

NO and axonal degeneration

Axonal degeneration has long been recognised to be an important structural correlate of clinical deficit in MS.[40] Because degeneration is an irreversible process, axonal protection in patients with MS is a major therapeutic goal, and understanding of the mechanisms involved is important.

Within MS lesions axonal injury occurs in two stages. Extensive axonal injury is present during the stage of acute inflammatory demyelination,[116,117] but there is also a "slow-burning" axonal degeneration in chronic inactive demyelinated plaques.[118] The mechanisms underlying the degeneration are not understood in any detail in either type of lesion, but many more mechanisms have been advanced as candidates to account for degeneration in the inflammatory lesions than for the putatively "inactive" lesions. This review focuses only on the potential mechanisms that involve NO.

The extent of axonal destruction in MS lesions is partly related to the number of cytotoxic T cells,[119] but especially to the number of activated macrophages[117] that can be found in close apposition with injured axons.[116] Thus, inflammatory mediators, such as NO, produced by activated macrophages and cytotoxic T lymphocytes may be the driving force for axonal injury. Although axonal injury is most prominent in inflammatory demyelinating lesions, we suggest that factors associated with inflammation may also contribute to the slow rate of degeneration observed in chronic lesions. Although it is sometimes believed that chronic MS lesions lack a noticeable inflammatory component, this is not true, and even in chronic inactive lesions axonal degeneration occurs on a background of residual inflammation[120] associated with signs of microglial activation. We emphasise these considerations because there is a significant correlation between the extent of axonal injury and the extent of inflammation both in the overall sample of all MS plaques at

different stages of demyelinating activity, and also when inactive lesions are analysed separately.[118] This observation indicates that inflammation is associated with axonal injury not only in actively demyelinating lesions, but also in "burnt out", inactive ones.

For the mechanism of axonal degeneration, an initial injury seems to be followed by downstream events mediated by an influx of calcium ions into the axoplasm. The influx probably results in the activation of axonal proteases and disruption of the axonal cytoskeleton[105] in a way similar to that described for axonal degeneration in ischaemia or traumatic brain lesions.[121–123] What causes the initial influx of calcium ions is not known, but a recent finding suggests that it might occur as a result of sustained electrical activity in axons, if this occurs while the axons are exposed to the low micromolar concentrations of NO that might be expected at a site of inflammation (figure 3).[124] Although the conclusion that this finding accounts for some axonal degeneration in MS would be premature, clearly the combination of impulse activity and NO exposure will occur in active MS lesions. The underlying mechanisms have not yet been studied in detail, but calcium is likely to enter the axons through reverse operation of the sodium–calcium exchanger after a rise in intra-axonal sodium,[121–123] due, in turn, to NO-mediated mitochondrial inhibition (which can be profound).[125,126] We have examined whether it is possible to protect axons from the effects of NO with blockers of either sodium channels or the sodium–calcium exchanger, as we discuss below in the context of a potential therapy.

Apart from a potential role for electrical activity in axonal degeneration, the simple exposure of axons to low micromolar NO can result in persistent conduction block[127] due (at least in vitro) to axonal degeneration.[83,128] Again, mitochondrial inhibition due to NO may play a part. We have recently investigated mitochondrial ATPase expression in MS lesions and have found a pronounced reduction in the axonal density of ATPase-positive mitochondria in a subset of acutely demyelinating plaques (HL, unpublished; figure 4).

NO may also be involved in another way, namely by releasing active forms of matrix metalloproteinases: it is easy to imagine that such proteases might play a role in damaging axons,[129] especially demyelinated ones.

NO and grey-matter pathology in MS

MS is generally regarded to be a disease affecting the white matter of the CNS, but there can also be very substantial involvement of the grey matter and, in particular, the cortex.[110,111,130] Although macrophage infiltration and microglial activation are much less prominent in cortical lesions than in white-matter plaques, both the demyelination and the axonal and neuronal damage occur in close association with these cells. Demyelination of cortical axons is the feature defining cortical plaques, but there is also a profound axonal injury, associated with apoptosis of nerve cell bodies.[111] The apoptotic stimulus is not yet known, but neurons can be induced to undergo apoptosis in vitro when exposed to low concentrations of NO,[131,132] and they are more sensitive to NO in this respect (and many others) than astrocytes.[133] Apoptosis can also be induced by exposure to peroxynitrite, and it is interesting that certain trophic factors can provide protection,[134] because neurons might become starved of trophic factors derived from glial cells if their axons suffer demyelination. Apart from apoptosis, exposure of neurons to higher NO concentrations results in necrosis;[132,134] the several NO-mediated mechanisms that can conspire to kill neurons and other cells have been reviewed elsewhere.[3–5,135] Several of the likely effects of NO in MS are summarised in figure 5.

NO-based therapy for MS

From the information presented in this review, it might appear that an effective therapy for MS could result from the inhibition of NO production, especially the inhibition of iNOS. However, over the past 10 years around 40 investigations have examined the role of NO in experimental autoimmune encephalomyelitis, but no clear picture has emerged. For example, whereas some studies have found that aminoguanidine (a partially selective inhibitor for iNOS) inhibits disease expression in a dose-dependent manner,[136] other studies have found that NOS inhibition can be deleterious, to the extent of inducing particularly severe disease in rat strains that are normally resistant to the induction of experimental autoimmune encephalomyelitis.[137] The effects of aminoguanidine treatment can depend partly on the timing of its administration, and one study found that early treatment was beneficial, whereas late treatment was deleterious; these findings suggest that the effects of iNOS inhibition may differ in the

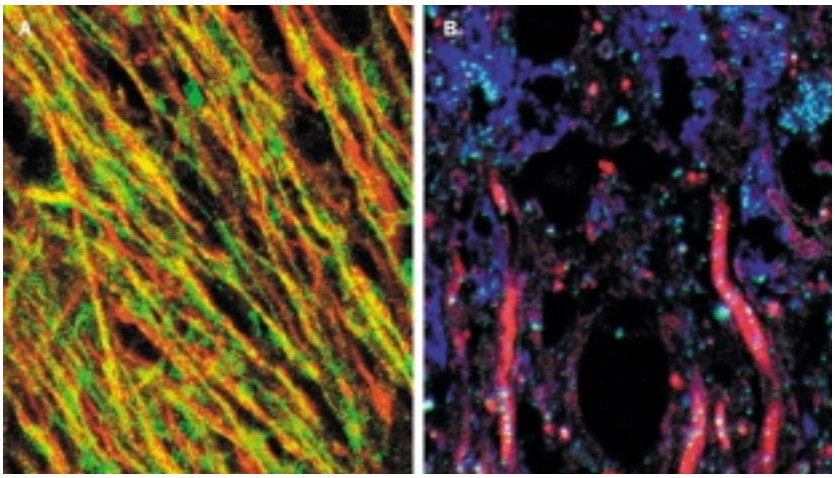

Figure 4. Staining of ATPase in normal white matter (A) and in an actively demyelinating plaque of acute MS (B). The confocal laser images show ATPase in green/yellow, neurofilament in red, and macrophages (positive for HLA DR) in blue (macrophages are absent in the control tissue). In the MS tissue there is a major reduction of ATPase immunoreactivity, and most of the reactivity is present in the macrophages; there are only few mitochondria in the axons and the rest of the tissue.

induction and progression phases of experimental autoimmune encephalomyelitis.[138] However, such a simple explanation fails to take account of all the disparate findings from the different studies. For example, mice lacking the iNOS gene develop more severe experimental autoimmune encephalomyelitis but less severe experimental autoimmune uveoretinitis than wild-type controls.[139,140] Other factors that seem to play a part in determining the consequences of NOS inhibition are the type of experimental autoimmune encephalomyelitis studied,[141] the dose and specificity of the NOS inhibitor,[7] its ability to penetrate the CNS, and the sex and strain of the rat model. Detailed discussion of the different findings is beyond the scope of this review, but interested readers are referred to a very helpful chronological account,[142] (see also 143) which tentatively concludes that, in experimental autoimmune encephalomyelitis, increased NO in the target tissue is detrimental in many cases, whereas increased NO during immunisation can be partly beneficial by controlling and limiting the immune response.

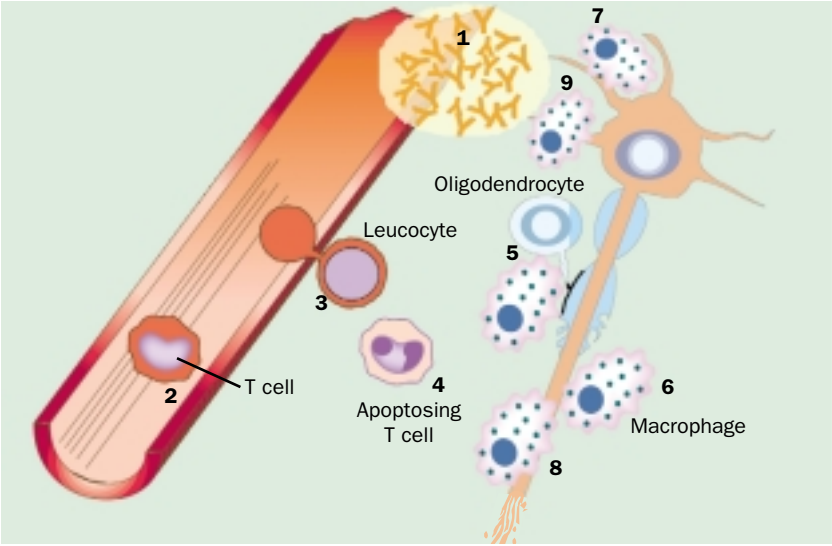

Figure 5. NO may act at several steps of the inflammatory cascade both to potentiate and to ameliorate the pathology of inflammatory demyelinating lesions. NO can disturb the permeability of the BBB and facilitate antibody leakage into the lesions (1), and it may inhibit T-cell activation in the blood and lymphatic tissue thereby inhibiting T-cell mediated inflammation (2). NO may inhibit the expression of adhesion molecules at the BBB and thereby impair the recruitment of inflammatory cells into the lesion (3), and it may also augment the apoptosis of T lymphocytes in the CNS, contributing to the termination of the inflammatory reaction (4). The NO produced by iNOS-positive inflammatory cells may destroy oligodendrocytes and/or damage myelin resulting in demyelination (5), and it may also block conduction in demyelinated axons, thereby contributing to the loss of function (6): function may also be impaired by effects of NO on synaptic transmission (7). NO may also directly damage axons (8) and, within the grey matter, neurons (9).

Apart from studies based on NOS inhibition, other studies have found that inhibitors of type IV phosphodiesterase can suppress experimental autoimmune encephalomyelitis,[144] and their mechanism of action may involve NO.[145] Similarly, linomide protects animals from experimental autoimmune encephalomyelitis, and this effect has been attributed to its ability to inhibit NO production by glial cells.[146] Uric acid administration is also an effective therapy for experimental autoimmune encephalomyelitis,[58,147] and the fact that it can act as a scavenger for peroxynitrite (in addition to some other reactive species) may be relevant. Patients with MS are reported to have significantly lower serum concentrations of uric acid than healthy individuals.[147] Indeed, MS and hyperuricaemic gout seem to be almost mutually exclusive conditions; thus, hyperuricaemia may protect against MS.[147]

To reduce the damage caused by NO, there may be an alternative to limitation of NO production. Several of the deleterious effects of NO may actually be mediated by peroxynitrite, which, as we said earlier, is formed from the combination of NO and superoxide. Therefore, it may be possible to limit peroxynitrite formation by restricting the production of superoxide rather than NO. The rather inconsistent results obtained with NO inhibition in experimental autoimmune encephalomyelitis can therefore be balanced against the rather more consistent findings with therapies designed to limit superoxide concentrations.[4] Reduction of superoxide concentration would leave intact the beneficial effects of NO production, and might also limit the formation of hydrogen peroxide, which can be toxic to oligodendrocytes (at least in culture).[148,149]

With regard to axonal protection, recent evidence indicates that degeneration due to the effects of NO can be prevented by the use of inhibitors of either sodium channels[83,128] or the sodium–calcium exchanger.[128] For example, a low dose of the sodium-channel blocking agent flecainide protected axons from degeneration resulting from sustained electrical activity experienced in the presence of NO.[128] Sodium-channel blocking agents are already in widespread clinical use for different purposes, and our initial findings indicate that this approach may provide axonal protection in experimental autoimmune encephalomyelitis (Bechtold D, Kapoor R, and Smith KJ, unpublished).

Conclusion

In summary, the role of NO in MS is likely to be much more complicated than originally thought, with a complex interplay between largely deleterious direct effects on neurological tissues and broadly beneficial regulatory effects on the immune system. We are not aware of any current clinical trials in MS based (deliberately at least) on the modulation of NO, perhaps because of concern about disturbing the beneficial effects. However, it would be wrong to conclude that because the adverse and beneficial effects of NO are partly compensatory, NO has only a small role in MS. Rather, it seems that the role of NO in MS is substantial, and each of the compensatory forces is large. Thus, in a disease such as MS, in which different lesions may

Search strategy

Data for this review were selected following searches of Medline and pre-Medline databases. Additional references were selected from relevant articles and the authors' own files. Only papers published in English were reviewed. The original number of references was reduced substantially to 150 on request from the editor.

concurrently be at different stages of development and experiencing different phases of immune regulation, an instrument as "simple" as iNOS inhibition is unlikely to result in predictable results, and the consequences may be severe (as they can be in experimental autoimmune encephalomyelitis). Furthermore, iNOS inhibition might also render the patient more vulnerable to invading micro-organisms and tumours. To avoid such consequences, the reduction of superoxide concentrations could be one approach, but an approach that combines iNOS inhibition with an immunomodulatory therapy is another possibility. This combination would compensate for the loss of the immunomodulatory effect of NO. Interestingly, it seems that this combination of events has been (serendipitously) achieved in one of the most effective existing therapies for MS—interferon beta. Several studies have found that this cytokine is not only an immune modulator, but also an inhibitor of iNOS expression in human astrocytes.[150] Perhaps the efficacy achieved with interferon beta could be surpassed if a more effective inhibitor of iNOS were combined with a more effective immunomodulatory compound.

Authors' contributions

Both authors contributed equally to this collaborative effort.

Conflict of interest

We have no conflict of interest.

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