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The multiple sclerosis lesion: initiated by a localized hypoperfusion in a central nervous system where mechanisms allowing leukocyte infiltration are readily upregulated?

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Abstract — A mechanism is proposed that may explain the factors that initiate a multiple sclerosis (MS) lesion. It is based upon the following two hypotheses: (i) there is a lower stimulus threshold for upregulating the mechanisms that result in leukocyte infiltration in individuals predisposed to developing MS; (ii) the MS lesion is initiated as a reduction in blood flow to a localized region of white matter. This reduction in blood flow leads to: (a) degenerative white matter changes affecting oligodendrocytes; (b) upregulation of chemokines in the endothelial cells and/or glial cells; and (c) upregulation of cell adhesion molecules on endothelial cells. Signals from the hypoxemic and hypoglycemic glial cells, likely involving myelin molecules and cytokines, result in an inflammatory immune response that results in rampant demyelination. Evidence supporting the proposed mechanism is presented, as well as suggestions on how to test the validity of the proposal.

Consequences of hypoperfusion in the central nervous system

In vitro experiments arising in this laboratory demonstrate that the oligodendroglial lineage of cells is readily damaged by a number of environmental perturbations including elevated temperature, hypoxia, hypoglycemia and oxidative stress (1–4). The cells die because they have increased ability to generate reactive oxygen species while at the same time having a poorer ability to scavenge them (5). These results led to the question of what are the possible effects of having localized Raynaud's-like (6) hypoperfusion in the central nervous system (CNS). What effect could one expect if blood flow is reduced by 25–50%? Hypoperfusion would result in hypoxemia, hypoglycemia and possibly localized increases in temperature due to poorer ability to dissipate heat, conditions that have been shown to be detrimental to the oligodendroglial lineage in vitro.

Cerebral white matter has been demonstrated to be highly vulnerable to focal ischemic insults with white matter changes being seen as early as neuronal damage is seen (7). Less drastic changes in cerebral

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blood flow have been demonstrated to preferentially affect white matter. Demyelinating lesions with little effect on grey matter arise in rats and gerbils where the cerebral hemispheres were exposed to chronic hypoperfusion and where blood flow was reduced by 25–50% (8,9). Hypoperfusion caused demyelination, upregulation of MHC I expression by microglia within 24 h and, by 3 days, there was increased microglial expression of MHC II antigens (9). By 7 days, there was increased immunoreactivity for glial fibrillary acidic protein in the astrocytes. Loss of myelin was related to extent of glial activation. Glial activation and white matter changes were most marked in the optic nerve and tract. White matter changes, as well as microglial activation, can be suppressed by treating the animals with the cyclophosphamide (10), suggestive of some immune involvement in the degenerative changes. There is now accumulating an abundance of evidence that the immune system plays a major role in delineating the extent of damage seen following an ischemic insult, e.g. (11).

Specific damage to white matter with little or no involvement of grey matter is also seen in human patients who have prolonged periods of hypoxia with attendant metabolic acidosis and hypotension (12,13). White matter changes involving demyelination are also seen in elderly human patients subjected to hypoperfusion due to intermittent periods of hypotension (14) or due to Binswanger's disease (15).

Although there is clear evidence that there is an immune involvement in the damage that develops following a severe ischemic insult with massive leukocyte infiltration of the affected tissue, there is much less involvement of the immune system where there is relatively moderate reduction in blood flow such as is seen in conditions of hypoperfusion. Leukocyte infiltration of brain parenchyma requires an upregulation of a number of specific cell adhesion molecules on the endothelial cells (16,21,22). There is now ample evidence that ischemic insults of sufficient severity can cause upregulation of cell adhesion molecules on endothelial cells, thus allowing infiltration of leukocytes into the brain parenchyma resulting in an inflammatory lesion (11,23,24). Signals that cause leukocyte chemotaxis and expression of cell adhesion molecules on the endothelium may come directly from the endothelial cell or from glial cells. Hypoxia has been demonstrated to cause endothelial cells to express and secrete interleukin 8 (IL-8) and other chemokines (25). Cytokines have also been demonstrated to be produced by glial cells (22). That significant leukocyte infiltration is not seen following moderate hypoperfusion may simply be due to there being insufficient upregulation of cell adhesion molecules on the endothelial cells.

The multiple sclerosis lesion

In an MS lesion, there is myelin breakdown accompanied by an inflammatory reaction (16,17). The inflammatory lesion is usually thought to be T-cell driven (18) with the massive demyelination believed to be due to 'bystander' effects (19). The effectiveness of interferon-β treatment in reducing the incidence of lesion development suggests that the symptoms of MS are due mainly to the immune attack on nervous tissue (20).

A major question in the development of an MS lesion that is still unresolved is whether the inflammatory environment of an MS lesion is what causes the initial myelin breakdown or whether initial myelin breakdown results in inflammation that in turn exacerbates the demyelination process. The earliest detectable event in the development of a lesion is an increase in the permeability of the blood–brain barrier followed by inflammation and demyelination (26). Rodriguez and colleagues (27,28) suggest that myelin breakdown is what initiates the inflammatory response. Their evidence for this conclusion is: (i) electron microscopic studies on early acute MS lesions previously identified with magnetic resonance imaging (MRI) demonstrate that the earliest observable change is degeneration within the inner oligodendroglial cytoplasmic loops and widening of the inner lamellae; and (ii) in some lesions there is demyelination with minimal lymphocyte infiltration. These early changes are followed by rampant demyelination that is coincident with infiltration of inflammatory cells.

The results of the hypoperfusion studies from both animals and humans suggest that myelin breakdown is insufficient to result in the establishment of an inflammatory lesion. Also, the presence of cell adhesion molecules and associated cytokines is not sufficient to result in an MS-like lesions (22). It appears that *both* myelin breakdown as well as upregulation of mechanisms that allow leukocyte infiltration are necessary for the development of an MS lesion.

Hypotheses

1. The factor that may predispose individuals to developing MS is a lower stimulus threshold for upregulating the mechanisms that result in leukocyte infiltration.
2. The primary insult that initiates an MS lesion is a reduction in blood flow to a localized region of the CNS. This reduction in blood flow leads to: (a) degenerative white matter changes affecting oligodendrocytes and possibly other cell types; (b) upregulation of chemokines in the endothelial

cells and/or glial cells; and (c) upregulation of cell adhesion molecules on endothelial cells.

A signal or signals from the hypoxemic and hypoglycemic glial cells, likely involving myelin molecules and cytokines, results in an inflammatory immune response that gives rise to rampant demyelination.

Evidence supporting the hypotheses

Several proton magnetic resonance spectroscopy studies have demonstrated increases in lactic acid content of early active MS lesions (29,30). This increase in lactate may be a reflection of the inflammatory environment; however, one of the studies also demonstrated an increase in lactic acid in normal-appearing white matter adjacent to the MS lesions (29) suggesting that this may be an effect of hypoxemia.

There are a number of indications that vascular changes may be primary in MS. These include observations such as: (i) increased permeability of the blood–brain barrier without a necessary accompanying demyelination (31); and (ii) the presence of perivenular abnormalities characteristic of MS lesions in the retina of MS patients (32). The retina is a region free of myelin. Furthermore, an allergic encephalitis that has many resemblances to MS can be induced in guinea pigs with cerebral endothelial cell membranes (33). Such observations have resulted in the development of an hypothesis that the cause of MS is a focal Binswanger-like hypertension of genetically susceptible vessels that leads to vascular damage ultimately resulting in ischemia (34). The present hypothesis differs in that it is hypothesized that the MS lesion is a consequence of focalized hypoperfusion coupled with an increased ability to upregulate the mechanisms that result in leukocyte infiltration of the brain parenchyma.

The adhesion molecule ICAM-1 has been described as being upregulated on the endothelial cells not only within an MS lesion but also in non-lesion white matter from MS patients (35); this is suggestive of a readier upregulation of this molecule in the MS patient.

Possible roles of genetics and the environment in such a model

There is a significant genetic component to MS (36) with loci on five different chromosomes being implicated in several different recent studies (37). The specific genes affected in these loci are not yet known. Possibly some of the implicated genes may be related to how readily blood vessels vasodilate or vasoconstrict in response to signals; alternatively, they may be related to mechanisms that give rise to vasoactive molecules. Current studies on regulation of CNS microcirculation has focused on grey matter where there is a high degree of autoregulation of blood flow. Little is known about regulation of blood flow in white matter. The stochastic nature of release of signals regulating blood vessel caliber or of receptor number in the blood vessel smooth muscle cells may result in individuals predisposed to MS having areas of the CNS white matter being periodically hypoperfused. Other genes that may play a role in governing predisposition to MS may be concerned, in part, with governing the mechanisms that allowing leukocyte infiltration and activation.

There is also a significant environmental component to MS (38). The nature of the environmental component is not known but may include childhood infections, dietary trace element deficiencies, toxic components, etc. Such a factor, or absence of a factor, could conceivably have a multitude of effects that enhance the probability of development of demyelinating lesions. These could include, amongst others, the following: (i) subtle changes in the noncellular composition of the wall of blood vessels that ultimately affect the regulation of blood perfusion; (ii) permanent epigenetic changes in the ability of blood vessels to respond to vasoactive compounds; (iii) permanent epigenetic changes in endothelial cells that increase the probability of expressing cell adhesion molecules or chemokines; (iv) permanent epigenetic changes in glial cells that make them more susceptible to hypoxia and hypoglycemia; (v) permanent epigenetic changes that increase the probability of glial cells synthesizing and releasing inflammatory cytokines.

Tests of the hypotheses

When a cell experiences hypoxia, there is induction of a specific transcription factor known as hypoxia-inducible factor 1 (39,40). The presence of hypoxia-inducible factor 1 in discrete areas of prelesion white matter as well as early MS lesions would lend support to the mechanism of MS lesion development proposed in this manuscript. Cells experiencing hypoglycemia upregulate the expression of several glucose-regulated proteins (41), the upregulation of such glucose-regulated proteins would support the hypothesis that hypoperfusion is involved. Positron emission tomography (PET) scans for blood flow patterns may pick up foci of decreased blood flow that ultimately develop into full-blown lesions.

Demonstration of readier upregulation of cell adhesion molecules in endothelium in individuals with MS than in individuals who do not develop MS would also be supportive of the proposed mechanisms underlying the development of MS lesions. Of particular interest would be examination of the brains of MS patients who have died of non-MS-related causes. The intensity and pattern of cell adhesion molecule expression in response to a variety of insults may offer support for or against the mechanisms proposed in this manuscript. Hypothesis 1 would predict that there would be a stronger and more widespread upregulation of cell adhesion molecules in the endothelium of MS patients than of non-MS patients who have died because of similar neurological insults. Furthermore, if there is a readier upregulation of mechanisms that facilitate leukocyte infiltration into the CNS parenchyma, one would expect that individuals with MS would, on the average, experience greater brain damage following equivalent strokes.

Conclusion

If the MS lesion is initiated because of localized hypoperfusion in CNS tissue that is predisposed to readily upregulate the mechanisms that allow leukocyte infiltration, then lesions may be prevented from occurring by promoting CNS perfusion.

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