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The contribution of inflammation to acute and chronic neurodegeneration

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Neurons of the central nervous system (CNS) of mammals are terminally differentiated cells, and there is no endogenous capacity of the CNS to replace lost cells. Although there are circumstances in which transplantation of embryonic cells, or genetically engineered cells, can be used to replace cells that have degenerated, this is currently a highly experimental procedure, and it is likely to be a long time before it is in routine use. Following the adage that prevention is better than cure, developing strategies that prevent neuronal loss in the first instance is likely to be a valuable approach. Thus, attempts to prevent neuronal degeneration following an ischaemic insult or in chronic neurodegenerative diseases such as motorneurone disease and Alzheimer's disease, are important areas of neuroscience research. In recent years, it has been recognized that in many diseases of the CNS, neuronal cell loss is accompanied by an inflammatory response (Perry *et al.*, 1995). For example, following a stroke, leucocytes are recruited to the region of the lesion (Clark and Zivin, 1996) and in Alzheimer's disease pathology there are large numbers of activated microglia, the resident macrophages of the CNS (McGeer *et al.*, 1993). The key question that confronts the neurobiologist is whether the inflammatory response is only a consequence of tissue degeneration, or whether it also contributes to the outcome of the pathology. To understand the mechanisms by which inflammation may contribute to these diseases, we have to turn to experimental models of acute and chronic neuronal degeneration.

Acute neuronal degeneration

A widely used model of acute neuronal degeneration is the injection of a glutamate agonist into the parenchyma of the brain. The rationale behind this model is that there is good evidence that neuronal degeneration around the core of an ischaemic lesion, caused by a focal thrombosis for example, is caused by the release of glutamate from dying cells. This excess glutamate is not adequately taken up by compromised neurons and glia and literally excites the neurons to death, the so-called excitotoxic hypothesis (Choi, 1992).

Injection of the glutamate agonist kainic acid into the dorsal hippocampus of the mouse brain results in rapid degeneration of the pyramidal neurons (Andersson *et al.*, 1991). However, despite this rapid cellular degeneration, there is little sign of a typical inflammatory response. There is no obvious vascular response or neutrophil recruitment, such as would be seen following comparable cellular degeneration in a peripheral tissue. Monocytes are only recruited after a delay of several days. An obvious possibility to account for the paucity of leucocyte recruitment is that the endothelium fails to express the appropriate adhesion molecules or that the presence of the blood-brain barrier (BBB) prevents signals passing from tissue to the lumenal face of the endothelium. Such signals might involve the passage of a chemokine, for example, to be presented to the adhering leucocytes. However, these are not likely explanations, since CNS endothelium expresses the appropriate adhesion molecules (Bell and Perry, 1995) and kainic acid, by some unknown mechanisms, damages the BBB directly (Andersson *et al.*, 1991), yet the leucocytes are still not recruited. In addition, we have shown that a C-X-C chemokine delivered within the CNS parenchyma will readily recruit neutrophils to the brain in large numbers, showing that in the presence of the appropriate stimulus, the CNS endothelium is permissive

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for neutrophil extravasation (Bell *et al.*, 1996). It is still unclear why relatively few neutrophils are recruited to an acute excitotoxic lesion.

Associated with the neuronal degeneration induced by kainic acid injection is the rapid activation of the resident brain macrophages, the microglia. The microglia are normally quiescent cells, but are highly responsive to almost any local disturbance of brain homeostasis or neuronal degeneration (Kreutzberg, 1996). When microglia are activated, they upregulate their expression of a variety of cell surface antigens and may release inflammatory mediators. The question then arises as to whether the plethora of potential molecules that can be released by mononuclear phagocytes are indeed released by activated microglia and whether any of these molecules play a part in neurodegeneration or neuroprotection.

Recent studies by Strickland and colleagues (Tsirka *et al.*, 1995, 1997) show that tissue plasminogen activator (tPA) released by microglia may play a critical role in kainic-acid-mediated neurodegeneration. They found that "knockout" mice lacking tPA are resistant to kainic-acid-induced neuronal degeneration. The sequence of events that emerged from their work (reviewed in Anthony and Perry, 1998) is that the glutamate agonist induces the release of tPA from microglia and neurons, and the tPA converts plasminogen to plasmin. The substrate, or one of the substrates, for the plasmin was the laminin-rich extracellular matrix surrounding the neurons. The notion that disruption of the neuronal-extracellular matrix interactions is central to neuronal degeneration was supported by experiments in which microinjection of anti-laminin antibodies into the hippocampus of tPA-deficient mice was sufficient to restore kainic-acid-inducible neuronal degeneration. Thus, disturbances of the brain extracellular matrix mediated via microglia may be an important and previously unsuspected component of neuronal degeneration. Microglia secrete not only tPA, but also matrix metalloproteases (MMPs) which can play a part in the proteolytic cascade and the degradation of the extracellular matrix (Anthony *et al.*, 1997a).

The observations have important implications not only for our understanding of the mechanisms by which activated macrophages might contribute to acute neuronal degeneration, but also for a current therapeutic intervention for ischaemic stroke. tPA, if administered within 3 h of an ischaemic stroke, will provide benefit for patients (Marler *et al.*, 1995). However, if tPA enters the CNS, this obviously has the potential to exacerbate neuronal degeneration.

In contrast to the kainic acid lesion where there is no direct damage to the brain vasculature, in a stroke or traumatic lesion to the brain the vasculature of the brain is directly damaged. Associated with these lesions, there is a conspicuous infiltrate of neutrophils and there is an increasing body of evidence to show that neutrophil depletion or inhibition of neutrophil recruitment will have beneficial effects on the lesion outcome (Clark and Zivin, 1996; Clark *et al.*, 1996; Carlos *et al.*, 1997). The mechanisms by which the neutrophils exacerbate the lesion are multiple; they may further damage the BBB and promote oedema, and the release of free radicals and various proteases is likely to result in direct damage to neural tissue.

We have examined how neutrophils might damage the BBB in a model where only neutrophils are recruited to the CNS parenchyma. Injection of interleukin-1β (IL1β) into the parenchyma of juvenile rat brain results in the rapid recruitment of neutrophils and breakdown of the BBB, as measured by the extravasation of intravenously delivered horseradish peroxidase (HRP) (Anthony *et al.*, 1997b). The IL1β injections into neutropenic animals failed to produce HRP extravasation, indicating that neutrophil adhesion and/or extravasation was the key element in BBB breakdown. When the neutrophils bind to the endothelium, we see by immunocytochemistry phosphotyrosine staining in the endothelium and this is followed by loss of staining of the tight junction proteins occludin and ZO-1 (Bolton *et al.*, 1998). We interpret these data as showing that neutrophil binding to the CNS endothelium results in a signalling cascade in the endothelial cell that in turn results in dissolution of the tight junction complex and breakdown of the BBB. *In vitro* studies with brain endothelial cells show that cross-linking of cell surface ICAM-1 results in phosphorylation of the cytoskeleton-associated protein cortactin (Durieu-Trautmann *et al.*, 1994). The neutrophils, having crossed the BBB, enter the CNS parenchyma. However, the molecular mechanisms by which neutrophils might then contribute to neuronal degeneration have been poorly studied, but recent evidence implicates MMP-9 secretion by neutrophils (Romanic *et al.*, 1998).

APP = amyloid precursor protein.
BBB = blood-brain barrier.
CNS = central nervous system.
HRP = horseradish peroxidase.
ICAM = intercellular adhesion molecule.
IL = interleukin.
MMP = matrix metalloprotease.
NSAID = non-steroidal anti-inflammatory drug.
PrP = prion protein.
tPA = tissue plasminogen activator.

Chronic neurodegeneration

The chronic neurodegenerative diseases such as Parkinson's disease, Alzheimer's disease and motor-neurone disease evolve over many years, likely decades. The inflammatory response associated with these diseases is highly atypical and dominated by cells of the mononuclear phagocyte lineage, the microglia (McGeer *et al.*, 1993). It is unclear whether all of these cells are derived from the resident microglia, or whether there is also an influx of monocytes which subsequently adopt the morphology and phenotype of microglia. The microglia in regions of pathology express raised levels of MHC class II and other plasma membrane molecules. There is also evidence for the activation of the complement cascade and deposition of membrane attack complex in the regions of cortical pathology in the Alzheimer's diseased brain (Webster *et al.*, 1997). Observations such as these suggest that there is an ongoing inflammatory response in Alzheimer's disease and also other chronic neurodegenerative diseases. The problem as to whether this inflammation is a *consequence*, *contributory* or *causal* of the neurodegeneration has in part been answered by epidemiological studies. The evidence shows that persons taking non-steroidal anti-inflammatory drugs (NSAIDs) are in some way protected from either the onset of Alzheimer's disease, or its progression (Breitner, 1996). The mechanism of action of NSAIDs in the context of this disease is unclear and to study this in post-mortem brain material poses considerable problems. One route to the study of the inflammatory response would be to study laboratory models of chronic neurodegeneration. A number of transgenic mice have been developed in which the overexpression of the amyloid precursor protein (APP) leads to deposition of the amyloidogenic peptide Aβ (Price and Sisodia, 1998). However, the disease seen in the brains of these animals only partially resembles the pathology seen in Alzheimer's disease, and in particular there are no neurofibrillary tangles, limited, if any, neuronal death, and no conspicuous microglia activation.

Several authors have drawn attention to the similarities between Alzheimer's disease and prion disease (DeArmond, 1993). The obvious similarities involved are that both APP and the normal prion protein, PrP^c, are neuronal cell surface molecules. Both of these proteins are deposited in an amyloidogenic form and are associated with a chronic progressive neurodegenerative disease that spreads along defined anatomical pathways and results in synaptic loss, neuronal degeneration and cognitive decline. From the point of view of inflammation biology, both diseases involve the deposition of an amyloidogenic protein or peptide which has the capacity to activate microglia *in vitro*, which leads to the production of free radicals and possibly other neurotoxic molecules (Meda *et al.*, 1995; Brown *et al.*, 1996). Thus, we have been interested to learn about the inflammatory response in murine prion disease as a laboratory model of chronic neurodegenerative disease. It has been widely and often stated that prion disease is not associated with an inflammatory response and that there is no recognition of the disease process by the immune system (Prusiner, 1996). We have examined this issue in C57BL/6J mice infected with the ME7 prion agent, a well studied agent that has been passaged in mouse brain for several decades, that gives rise to a well defined disease time course and pathology (Betmouni *et al.*, 1996).

We established (Williams *et al.*, 1994) that in the brains of animals with terminal disease, there was conspicuous microglia activation, akin to what has been observed in human brain from patients with Alzheimer's disease or prion disease. The activated microglia are not only derived from proliferation of the resident microglia, but bone-marrow chimaeras have been used to demonstrate that some of the activated microglia were derived from infiltrating monocytes (Williams *et al.*, 1995). It is interesting to note that it was not possible to distinguish by immunocytochemistry, on morphological criteria or differential expression of macrophage antigens, which of the mononuclear phagocytes were derived from resident microglia and which were recently recruited monocytes.

The presence of activated microglia is not surprising, since it is well known that microglia are activated wherever there is neuronal degeneration (Kreutzberg, 1996). It is unclear whether the microglia are simply responding to neuronal degeneration, or whether they play a contributory role. We have thus investigated when the microglia first become activated, whether this happens prior to the neuronal degeneration or follows it. The injection of a brain homogenate containing the prion agent ME7 into the dorsal hippocampus of the mouse brain results in a non-specific acute inflammatory response that is indistinguishable from that seen following injection of a normal brain homogenate. This non-specific response resolves over the next few weeks and there is no evidence that the injected prion produces acute neuronal degeneration (Betmouni and Perry, 1998). At about eight weeks postinjection, the microglia in the region of the injection site become activated, as seen by their upregulation of several cell surface and cytoplasmic antigens, and the microglia activation is accompanied by an infiltrate of T lymphocytes that are predominantly $CD8^+$ cells (Betmouni *et al.*, 1996). The activated microglia and $CD8^+$ T lymphocytes appear in the dorsal hippocampus prior to onset of overt neuronal degeneration. Over the next weeks, the activated microglia, T lymphocytes and activated astrocytes appear in regions anatomically related to the injected region of the brain.

The presence of $CD8^+$ T lymphocytes is intriguing and at the present time their specificity is not known, but there are several points worth explaining. Firstly, mice lacking T lymphocytes still succumb to prion disease following an intracranial injection of prion agent (McFarlin *et al.*, 1971). This indicates that the T lymphocytes are monitoring some aspect of the neurodegenerative process without necessarily contributing to the progression of the disease. Secondly, $CD8^+$ T lymphocytes appear to enter the central nervous system in response to ongoing degeneration in situations other than prion disease. In mice with a spinal cord lesion, $CD8^+$ T lymphocytes are recruited to the sites distal to the lesion in regions where there is ongoing retrograde and Wallerian degeneration. The recruitment of $CD8^+$ T lymphocytes to the injured cord was influenced by whether the animals were raised in a conventional or SPF animal house (Schnell *et al.*, 1997). Thus, $CD8^+$ T lymphocytes appear to be recruited to sites of chronic neurodegeneration. The nature of the signals involved are unclear, but a plausible explanation would be that chronic neurodegeneration induces synthesis and secretion of a particular chemokine.

The early appearance of activated microglia indicates the presence of a subtle local disturbance of CNS homeostasis that is not reflected in neuronal loss until many weeks later. At the present time, we do not know what this disturbance may be, but it is likely to involve the deposition of protease-resistant prion protein, PrP^{sc}, which is likely to have the capacity to activate the microglia (Brown *et al.*, 1996). Whether these activated microglia contribute to the neuronal degeneration can be investigated by treatment of the animals with the relevant anti-inflammatory drugs.

An interesting question that arises from the finding of activated microglia early in the disease process is whether these animals show a behavioural deficit prior to the onset of neuronal degeneration at twenty weeks postinjection. Following bilateral injection of the ME7 prion agent into the dorsal hippocampus, mice show behavioural signs, hyperactivity, memory impairment, consistent with hippocampal damage prior to the onset of neuronal degeneration but at the time that there is microglia activation and T-lymphocyte recruitment (Betmouni *et al.*, 1998). The nature of pathology that underlies these deficits remains to be elucidated.

Conclusion

There is a growing body of evidence to implicate inflammation in acute and chronic neuronal degenerative diseases in the central nervous system. The evidence shows that the responses of inflammatory cells in acute and chronic neurodegeneration are distinct. Understanding the molecular mechanisms that result in the recruitment of different leucocytes and the molecular mechanisms by which they contribute to neuronal degeneration provides potential routes to both diagnosis and treatment of these diseases.

Key-words: CNS, Neurodegeneration; Alzheimer's disease, Parkinson's disease, Inflammation, Review.

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