Cytokines and adhesion molecules in acute brain injury

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Once thought to be relatively shielded from the immunological and inflammatory processes which occur in the tissues of other body systems after acute injury, the brain participates actively in these processes. As a result of trauma, haemorrhage or ischaemia, injury to the brain releases mediators such as the cytokines which activate inflammation and cause further secondary brain injury. Intensive care physicians can do little to alleviate the gravity of the primary injury, but by understanding the mechanisms responsible for secondary injury, and how these mechanisms may, in future, be altered by drug therapy, they may be able to improve patient management and outcome.

A primary brain injury stimulates the cells of the central nervous system (CNS) to produce a variety of mediators. Laboratory and human studies have shown that there are at least three important cytokines which are released both by microglia and astrocytes after injury: interleukin-1β (IL-1β), tumour necrosis factor α (TNFα) and interleukin-6 (IL-6). These proteins function as intercellular communication molecules and stimulate the reparative process which is termed gliosis. Gliosis, however, results in further production and release of cytokines by hypertrophied astrocytes and microglial cells, in addition to mediators released by cells of the peripheral immune system, such as polymorphonuclear cells (PMN), which migrate across a “leaky” blood–brain barrier. The net result may therefore be further damage to brain tissue. The first part of this review focuses on the parts played by these cytokines.

Leucocyte adhesion molecules, which are expressed on the surface of leucocytes and endothelial cells, control the migration of leucocytes into tissue. The expression of these molecules after brain injury is linked closely to cytokine production. They mediate toxicity in two ways: by causing leucocyte plugging of micro-vessels and by facilitating the release of toxic oxygen-derived free radicals by PMN which migrate into brain tissue as a result of adhesion molecule activity. The second part of this review focuses on the parts played by the adhesion molecules, intercellular adhesion molecule (ICAM)-1, E-selectin, L-selectin, P-selectin and the integrins in brain injury, and on the evidence linking the cytokines to adhesion molecule upregulation. Finally, we shall discuss if anti-cytokine or anti-adhesion molecule therapy may improve outcome after acute brain injury.

Cytokines

The cytokines are low-molecular weight polypeptides which are synthesized and released by multiple cell types throughout the body. Their actions are numerous but they act primarily as mediators of the inflammatory process (either pro- or anti-inflammatory) and as growth factors. Many of the clinical signs which are seen after an acute brain injury (e.g. pyrexia, neutrophilia and cerebral oedema, secondary to disruption of the blood–brain barrier) are believed to be caused by cytokine activity. Cytokines also stimulate the formation or release of many types of secondary mediators such as the oxygen-derived free radicals, neuropeptides and arachidonic acid derivatives, and upregulate the activity of the adhesion molecules. There is a great deal of overlap in the functions of these molecules, in particular between IL-1β and TNFα. Figure 1 summarizes the CNS actions of these cytokines.

INTERLEUKIN 1β AND TNFα

IL-1β and TNFα have similar local and systemic actions, which have been reviewed previously in the British Journal of Anaesthesia. Both cytokines have been implicated in the pathophysiology of disease states, including alcoholic liver disease, the systemic inflammatory response syndrome (SIRS), multiple trauma, burns and rheumatoid arthritis.

Experimental studies have shown that activity of both IL-1β and TNFα is increased within the first hours after a traumatic brain injury (TBI), and that microglia are a source of IL-1β, which is a potent stimulant of gliosis. Cerebral IL-1β and TNFα are increased acutely after cerebral ischaemia in animal stroke models, particularly in the first hours after injury. These findings suggest that IL-1β and TNFα are released very early after injury, and explain partly why these two cytokines are often not detected in serum in clinical studies of brain injury.

Several laboratory studies have demonstrated the toxic effects of IL-1β and TNFα, although some investigators have used doses of cytokines well in excess of physiological concentrations. IL-1β acts to stimulate production of other cytokines, including TNFα, and in particular IL-6, by both astrocytes and microglia. Similarly, TNFα causes the release of other cytokines, particularly IL-6.

Keywords: polypeptides, cytokines; blood, adhesion molecules; brain, injury

(Br. J. Anaesth. 1998; 80: 77–84)
There are fewer clinical studies which have implicated either IL-1β or TNFα in brain injury. IL-1β is increased in the cerebral cortex\(^7\) and IL-1β receptor concentration is increased in cerebrospinal fluid (CSF)\(^65\) after spontaneous subarachnoid haemorrhage (SAH). IL-1β is increased in the CSF of patients who have sustained a stroke\(^10\), while McClain and colleagues found increased IL-1β concentrations in ventricular fluid after a traumatic brain injury.\(^67\) Serum TNFα is increased in patients with meningococcal infection, and the increase is more marked in those who are hypotensive.\(^105\) Goodman and colleagues showed increased serum TNFα in 21 patients with traumatic brain injury, but serum concentrations did not correlate with outcome,\(^36\) while Ross and co-workers showed that TNFα was present in the serum of 18 of 50 patients with a TBI.\(^82\)

It is therefore apparent that both IL-1β and TNFα are important factors in the propagation of the inflammatory process in the early stages after a brain injury. We shall discuss the effects of IL-1β and TNFα on adhesion molecule expression later in this review.

**INTFLEUKIN-6**

IL-6 differs from IL-1β and TNFα in that it both causes and inhibits inflammation.\(^76\)\(^65\) Akira, Taka and Kishimoto have published a comprehensive review which covers its functions in detail.\(^4\) A particularly important function of IL-6 in brain injury is its action in increasing endothelial permeability.\(^88\) There has been some debate about whether or not IL-6 is an active mediator in the inflammatory process after tissue injury or is simply a marker of injury severity,\(^8\) but the evidence clearly implicates IL-6 in the pathophysiology of inflammatory brain injury.

Hariri and colleagues found increased IL-6 production by cultured human astrocytes after fluid percussion injury\(^\)\(^40\) and Shoahmi and colleagues showed increased IL-6 concentrations in contused rat brain, which peaked 8 h after injury.\(^94\) Other investigators showed similar increases in IL-6 in experimental trauma models,\(^102\)\(^108\)\(^111\) which usually lag behind the production peaks of IL-1β and TNFα.

There are many clinical studies which have investigated the role of IL-6 in brain injury. Osaka and colleagues investigated the theory that increased serum concentrations of IL-6 were related to brain tissue damage, and measured IL-6 concentrations in the serum of 70 patients after neurosurgery. They found an increase which peaked 24 h after surgery.\(^75\) In a similar study, Heesen and co-workers showed that serum concentrations of IL-6 were increased after craniotomy, peaking 24 h after operation.\(^41\) In 30 patients with a TBI, McClain and colleagues found increased serum concentrations of IL-6, which were highest on admission, together with greatly increased ventricular fluid IL-6. The decrease in serum IL-6 concentration correlated with clinical improvement, and concentrations decreased more rapidly in those with a Glasgow Coma Scale score greater than 8.\(^88\) A recent study of 22 patients with TBI found increased ventricular fluid concentrations of IL-6, and that there is an association between IL-6 concentration and production of nerve growth factor.\(^35\) Our group in Edinburgh measured arterial and jugular venous concentrations of IL-6 in 32 patients who had sustained either a TBI or spontaneous SAH and found increased systemic IL-6 concentrations, together with a marked jugular venous–arterial difference.\(^79\) This suggests significant intracranial production of IL-6 after brain injury, and supports the experimental work discussed earlier.\(^40\)\(^94\)

Clinical studies in non-traumatic brain injury further implicate IL-6 as a mediator in the secondary inflammatory response. IL-6 concentration is increased in the serum and CSF of stroke patients.\(^5\)\(^9\)\(^10\) IL-6 and IL-8 concentrations in CSF are increased after SAH, peaking on day 6.\(^67\)\(^65\) An interesting study by Amado and colleagues found that the concentration of IL-6 in patients’ plasma increases at the time of diagnosis of brain death.\(^3\)

As we have already discussed, IL-1β and TNFα, together with many other mediators, including platelet activating factor, interferon and the bradykinins, promote IL-6 production. The part played by IL-6 in the regulation of the intracranial inflammatory process is complex, because it has pro- and anti-inflammatory actions, and has a negative feedback effect on the production of IL-1β and TNFα,\(^45\) but it occupies a central point in the web of mediators which control the inflammatory response to brain injury.

**OTHER CYTOKINES**

There have been several other cytokines implicated in the secondary inflammatory response after brain injury. IL-8\(^76\)\(^84\)\(^91\) may function as a neutrophil chemo-
attractant (chemokine) after brain injury, a recent study showed that concentrations of IL-8 were increased in the CSF of patients who sustained a TBI, and that this was associated with nerve growth factor production. Expression of the macrophage inflammatory proteins MIP-1 and MIP-2 is increased after brain injury, and there is increasing interest in the physiological role of the growth factors, including transforming growth factor (TGF-β) and fibroblast growth factor (FGF) in the antagonism of the secondary inflammatory process.

Adhesion molecules

The leucocyte adhesion molecules are important control factors in the pathophysiology of secondary brain injury. There are three families of adhesion molecules. The first includes the glycoproteins, L-selectin, E-selectin and P-selectin. L-selectin is constitutively present on the surface of leucocytes, while E- and P-selectins are expressed on the endothelial cell surface after injury. P-selectin is also expressed on platelets. The glycoproteins mediate the initial tethering of leucocytes to the vessel wall by binding to counter-receptors within moments of tissue injury. Deficiency of either the selectins or their ligands causes failure of the migration process and recurrent infection.

The two other families mediate strong adhesion and migration across the endothelium into tissues. The immunoglobulin “superfamily” consists of ICAM-1, ICAM-2 and vascular cell adhesion molecule (VCAM) 1, which are present on the endothelial surface, and ICAM-3 which is present on the leucocyte cell surface. Their counter-receptors form the third family, the integrins (e.g. CD11b, CD18, Mac-1) (fig. 2). The blood of healthy humans contains soluble, active forms of the selectins and immunoglobulins (termed sL-selectin, sICAM-1).

This review focuses on those molecules which have been studied most closely in brain injury, namely ICAM-1, E- and L-selectin and the integrins. As the functions of the three families are similar, we shall consider them as a group for the remainder of this review.

Relationship between cytokines and adhesion molecules

Experimental work suggests that IL-1β, IL-6 and TNFα upregulate the activity of the adhesion molecules: one early study showed that treatment of endothelial cell cultures with IL-1β caused increased PMN adhesion by acting on endothelial cells. IL-1β increases ICAM-1 expression in fibroblasts, and IL-1β and TNFα increase ICAM-1 expression in hepatocyte cell lines. Hahne and colleagues discovered that TNFα induced expression of several types of adhesion molecule in mouse endothelioma cells: ICAM-1, VCAM-1, E-selectin and P-selectin. Kukielka and co-workers published findings on the relationship between IL-6 and upregulation of ICAM-1 in the pathophysiology of reperfusion injury in the myocardium. They showed that IL-6 mRNA expression was increased in a canine myocardial reperfusion model, and that this was followed by an increase in ICAM-1 expression in the same segments. In a clinical study which examined 22 patients admitted to the ICU with illnesses resulting from liver cirrhosis, leucocyte expression of the integrins CD11b and CD35 correlated with the concentration of IL-6 in plasma. It is apparent therefore that the cytokines are important regulators of adhesion molecule activity in non-neurological disease, and further experimental studies which examined brain tissue add weight to this important relationship in brain injury. TNFα and interferon-gamma cause induction of ICAM-1 expression in microglia, and administration of TNFα to cultured CNS endothelial cells causes increased expression of VCAM-1 and E-selectin. Similarly, IL-1β, TNFα, interferon-gamma and lipopolysaccharide (LPS or endotoxin) increase ICAM-1 expression. Rieckmann and colleagues found increased expression of ICAM-1 in human brain tissue sections after stimulation with TNFα and LPS, but Shrikant and co-workers were unable to show an effect of IL-6 on ICAM-1 expression in astrocytes or microglia. Although findings are occasionally contradictory, they strongly suggest that the increased concentrations of cytokines seen after acute brain injury upregulate the activity of adhesion molecules in the CNS, causing PMN infiltration and further cellular damage (fig. 3). In the next section we examine more closely the adhesion molecules, and the part they play in brain injury.

Adhesion molecules and brain injury

Clinical research into the role of adhesion molecules in disease is still in its infancy, particularly in acute brain injury, but many studies suggest that manipula-
tion of the effects of these molecules may prove to be a powerful tool in the treatment of inflammatory disease. A review of the adhesion molecules by Froese and colleagues can provide additional background information. The most extensively studied molecule is ICAM-1, a member of the immunoglobulin “superfamily”. This molecule is expressed on the vascular endothelium and, as has already been stated, facilitates strong adhesion and migration of white cells from vessels into tissue. There is also evidence of its role in the intra-parenchymal binding of leucocytes to tissue cells such as astrocytes in the CNS. The soluble forms of the adhesion molecules (e.g. sICAM-1) are those which can be measured by assay in the serum or plasma of normal individuals, but the part they play in the physiological response to injury is unclear. Increased concentrations of the soluble endothelial molecules (sICAM-1, sE-selectin) may result from damage to the endothelium as a result of systemic inflammatory processes. Alternatively, these same processes may cause “activation” of the endothelium, with upregulation of production and shedding of these molecules into blood. Investigators have explained changes in concentrations of the soluble form of L-selectin (one of the leucocyte bound molecules) in different ways, which are discussed below.

Laboratory studies add weight to the evidence provided by the small number of clinical studies on this subject in brain injury. PMN accumulate in damaged brain tissue and there is a significant correlation between the degree of PMN accumulation and the extent of cerebral oedema in rats. There is little laboratory work on this subject in TBI; most studies have examined adhesion molecules in stroke models, although this work is certainly relevant to all forms of cerebral ischaemia. Wang and Feuerstein showed, in a rat stroke model, an increase in cortical E-selectin expression by 6 h after injury, which peaked at 12 h and returned to basal concentrations by day 5. ICAM-1 expression is also increased 3 h after injury and remains increased for 5 days. Clark and colleagues studied CNS reperfusion injury in rats, with a temporary middle cerebral artery occlusion (MCAO) model. This important study showed an increase in cortical ICAM-1 expression from 1 h after injury. This correlated with moderate infiltration of PMN in the areas of ICAM-1 expression. There is an upregulation of cortical E-selectin after cerebral reperfusion injury in primates, and after SAH in rats there is increased expression of ICAM-1 on cerebrovascular endothelial cells. Another recent study showed that ICAM-1 deficient mice were less susceptible to cerebral ischaemia/reperfusion injury.

Many of the clinical studies on the subject of adhesion molecules carried out to date have been in the SIRS patient population. Cowley and co-workers found that serum concentrations of sICAM-1, sVCAM-1 and sE-selectin were increased in SIRS, particularly if there was multiple organ dysfunction (MOD). A high sE-selectin concentration correlates closely with MOD and death. Other studies also showed that sICAM-1 and sE-selectin were increased in SIRS (concentrations of sICAM-1 were significantly higher after 24 h in non-survivors than survivors). Patients with multiple trauma have also been studied: an increase in serum sICAM-1 after multiple trauma correlated with the severity of subsequent MOD.

There are only a small number of clinical studies which have investigated adhesion molecules in neurological disease and non-traumatic brain injury. ICAM-1 expression is increased in the cerebral vessels of patients with diagnoses of multiple sclerosis, viral encephalitis and cerebral infarct and there is a high concentration of sICAM-1 in the CSF of patients with bacterial meningitis, which correlates with measures of blood-brain barrier dysfunction. Kim and colleagues studied leucocyte integrin expression in patients with a diagnosis of stroke or transient ischaemic attack and found increased expression of CD11a and CD18. Lindsberg and co-workers studied brain sections of patients who had died from stroke disease and showed increased expression of endothelial ICAM-1.

There are no published studies to date which have investigated serial changes in adhesion molecules in TBI or spontaneous SAH, but Keski and colleagues...
reported that in a group of 153 patients with TBI there was a good correlation between injury grading and admission white cell count.\textsuperscript{45} In a recent stroke study, Fassbender and colleagues found increased sICAM-1 and reduced sL-selectin concentrations in patients with significant risk factors for stroke, and in addition found increased sICAM-1, reduced sL-selectin, increased sE-selectin and sVCAM-1 concentrations in a group who had sustained a stroke.\textsuperscript{26} This decrease in sL-selectin is explained by the notion that activated endothelial counter receptors “mop up” the “pool” of sL-selectin in serum. This theory supports the findings of Donnelly and colleagues.\textsuperscript{25} They found reduced sL-selectin concentrations in the serum of a subgroup of patients who progressed to develop the acute respiratory distress syndrome from a sample judged to be “at risk” on recruitment to the study. Blann and colleagues proposed a similar theory to explain their findings of a reduced concentration of sL-selectin in patients with systemic sclerosis and vasculitis. They also showed an inverse relationship between serum sL-selectin concentration and disease severity.\textsuperscript{10}

It is apparent therefore that acute brain injury causes increased expression of leucocyte adhesion molecules on cerebrovascular endothelium and in brain tissue itself, with expression mediated by cytokines. The final step in the investigative pathway is to examine the effects of cytokine and adhesion molecule antagonists on the process, and to examine if these compounds may potentially improve neurological outcome after brain injury.

**Anti-cytokine and anti-adhesion molecule therapy**

There are several questions to be answered in the final part of this review. What effects do anti-cytokine therapies have on cerebral tissues and expression of adhesion molecules after acute brain injury? Do monoclonal antibodies to adhesion molecules reduce secondary damage after a cerebral insult, and what effects do these potential therapies have in injuries to other organ systems? It is important, however, to consider that the cytokines function as natural communication molecules in host defence, and so antagonism of their actions may also have detrimental effects.

In experimental anti-cytokine therapy for brain injury, findings are encouraging. The increase in blood–brain barrier permeability and CSF white cell count caused by administration of intra-cisternal TNFα is abolished by treatment with a TNFα antibody.\textsuperscript{55} Two studies examined the effects of anti-cytokine therapy on cerebral oedema: in a rat MCAO model, administration of an IL-1 blocker reduced cerebral oedema\textsuperscript{101}; more recently, Shohami and co-workers showed that after TBI in the rat, administration of TNFα antibody caused a reduction in cerebral oedema and facilitated recovery of motor function.\textsuperscript{93}

IL-1ra shows promise as a neuroprotective agent. Professor Nancy Rothwell’s group in Manchester, provided what is probably the first direct evidence for cytokine involvement in brain injury when they showed that ischaemic and neurotoxic damage in the rat is inhibited by IL-1ra.\textsuperscript{78} In a later study they gave IL-1ra to rats who had sustained a TBI. In the group where treatment was given immediately after injury, a reduction in lesion size, assessed histologically, of 44% was found. Even if treatment was delayed by 4 h, a 28% reduction in lesion size was seen.\textsuperscript{103} Another approach to antagonizing the effects of IL-1β is to block the action of IL-1β converting enzyme (ICE), which converts the precursor molecule pro-IL-1β to its active form. Rothwell’s group showed that administration of an ICE inhibitor reduced ischaemic brain damage in a rat model.\textsuperscript{62}

Some workers have studied the effects of anti-cytokine therapy on adhesion molecule expression. In mice who have sustained a penetrating stab wound to the brain, treatment with antibody to IL-1 results in a significant reduction of ICAM-1 positive glia at 24 and 48 h after injury,\textsuperscript{92} and cytokine specific antibodies inhibit induction of ICAM-1 in adenocarcinoma cell lines by IL-1β and TNFα.\textsuperscript{85} The evidence would therefore suggest that anti-cytokine therapy is indeed capable of reducing cerebral tissue damage and adhesion molecule expression after brain injury.

Finally, we should consider the therapeutic potential of blocking the actions of the adhesion molecules themselves. This will of course antagonize a natural body defense mechanism, and may in some cases be detrimental, but it is theoretically a more attractive option than attempting to block the diverse actions of the cytokines, with their complex inter-relationships. Clark and Zivin have recently published a detailed review on this subject in stroke disease,\textsuperscript{74} but the subject has received little attention by investigators in TBI research.

Two groups have made substantial progress using models of CNS ischaemia. In 1991, Clark’s group from Oregon published two articles\textsuperscript{20,21} which examined the effects of anti-adhesion molecule antibodies in CNS ischaemia and showed that antibody to the integrin CD 18 and to the immunoglobulin ICAM-1 produced a significant reduction in neurological deficits in a model of spinal cord ischaemia. This implicates leucocytes in CNS reperfusion injury. Zhang’s group from Detroit has published findings from several studies in rat stroke models since 1994. They showed that antibody to various adhesion molecules reduces infarct size and PMN infiltrate.\textsuperscript{34, 44, 112}

In more recent work the group has looked at the effect of giving antibodies later than at the onset of ischaemia, which is much closer to the clinical situation. They showed that treatment with anti-integrin or anti-ICAM-1 antibodies several hours after the onset of ischaemia results in reduced infarct size.\textsuperscript{113} Other groups have reported similar benefits.\textsuperscript{36, 66}

Other antagonists may have a part to play in future anti-adhesion therapy: heparin oligosaccharides bind to L- and P-selectin and thus inhibit their binding to other counter-receptors,\textsuperscript{72} and inositol polyamines reduce binding of L- and P-selectin in vitro.\textsuperscript{13}

This evidence suggests that administration of agents which antagonize the harmful effects of neutrophil adhesion and migration into tissue may reduce secondary brain injury in humans, although again we must be aware that adhesion and migration of leucocytes are natural responses of the host to injury, and that antagonism of these actions may reduce the positive effects of the inflammatory response. Phase three trials of an anti-ICAM-1
antibody are currently in progress in patients with stroke disease, and we await the results with great interest.

Conclusion

Research to date strongly implicates cytokines such as IL-1β, IL-6 and TNFα, together with the leucocyte adhesion molecules, in the pathophysiological response to primary brain injury. The cascades or webs of mediators released after primary injury cause secondary brain injury which may be severe. Prevention of systemic hypoxaemia, hypotension and hypothermia, together with optimization of oxygen delivery to the brain are priorities in the current intensive care management of acute brain injury.

Despite improvements in pre-hospital and intensive care, mortality and morbidity caused by traumatic brain injury has not decreased substantially in the past decade. Further work in the areas of anti-cytokine and, in particular, anti-adhesion molecule therapy may result in the clinical use of these novel therapies, which may alleviate the devastating secondary sequelae of an acute injury to the brain.

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