CYTOKINES AND ACUTE NEURODEGENERATION

Stuart M. Allan and Nancy J. Rothwell

Cytokines have been implicated as mediators and inhibitors of diverse forms of neurodegeneration. They are induced in response to brain injury and have diverse actions that can cause, exacerbate, mediate and/or inhibit cellular injury and repair. Here we review evidence for the contribution of cytokines to acute neurodegeneration, focusing primarily on interleukin 1 (IL-1), tumour necrosis factor-α (TNFα) and transforming growth factor-β (TGFβ). TGFβ seems to exert primarily neuroprotective actions, whereas TNFα might contribute to neuronal injury and exert protective effects. IL-1 mediates ischaemic, excitotoxic and traumatic brain injury, probably through multiple actions on glia, neurons and the vasculature. Understanding cytokine action in acute neurodegeneration could lead to novel and effective therapeutic strategies, some of which are already in clinical trials.

CYTOKINES
In general terms, cytokines are proteins made by cells that affect the behaviour of other cells. They are produced mainly by the immune system.

MORBIDITY
The incidence or prevalence of a disease in a population.

ACUTE-PHASE PROTEINS
Molecules that are found in the blood shortly after an infection. They participate in early phases of host defence.

School of Biological Sciences, University of Manchester, 1.124 Stopford Building, Oxford Road, Manchester M13 9PT, UK.
Correspondence to S.M.A.
e-mail: stuart.allan@man.ac.uk

Cytokines have been implicated as mediators and modulators of diverse forms of neurodegeneration, and exert a variety of actions in the central nervous system (CNS). Here we review the contribution of CYTOKINES to acute neurodegeneration, our current knowledge about their mechanisms of action and some clinical implications of recent research.

Acute neurodegeneration

The term acute neurodegeneration describes clinical conditions in which neurons are rapidly damaged and usually die in response to a sudden insult. This description encompasses stroke, head injury, cerebral or subarachnoid haemorrhage, and ischaemic brain damage derived from fetal or perinatal hypoxia. These conditions cause massive MORBIDITY and mortality, and pose an enormous socio-economic burden. Although the type of insult and onset of neuronal injury is acute, subsequent neuronal loss can occur hours or days after the initial event (FIG. 1). This delayed cell damage results from endogenous factors that are released in response to the primary injury, which might be common to the clinical conditions outlined.

Inflammatory processes have been implicated in both acute and chronic neurodegenerative conditions. The CNS differs in its inflammatory response to other tissues. In general terms, cellular infiltration in the brain in response to inflammation, infection and injury is weaker and delayed[1], yet many inflammatory responses can be induced rapidly. These include the activation of microglia, and the expression and release of classical inflammatory mediators, such as ACUTE-PHASE PROTEINS, EICOSANOIDS, COMPLEMENT and cytokines[2].

Cytokines

Cytokines, a diverse group of polypeptides that are generally associated with inflammation, immune activation, and cell differentiation or death, include INTERLEUKINS (IL), INTERFERONS (IFN), tumour necrosis factors (TNF), CHEMOKINES and growth factors. They have diverse actions, and most have little or no known function in healthy tissues, but are rapidly induced in response to tissue injury, infection or inflammation. Their involvement in CNS disease is a rapidly growing area of biological and clinical research[2,3]. Because of the number of cytokines and the diversity of their actions, this review will focus primarily on the three cytokines that have been studied most extensively in the CNS — TNFα, IL-1 and transforming growth factor-β (TGFβ).

Many cytokines are produced as biologically inactive precursors. So, pro-TNFα must be cleaved by the enzyme TNFα convertase (TACE/ADAM17), which is

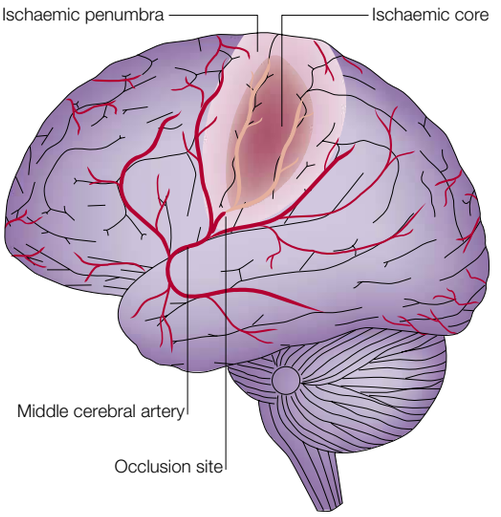


Figure 1 | **Damage after focal cerebral ischaemia.** Focal ischaemia can be induced by cerebral haemorrhage, embolism or brain injury, and results in rapid neuronal death in a core region within the immediate territory of the occluded artery. This is followed by a more delayed infarct (penumbral region), in which neurons might die many hours after the critical insult. Cytokines are expressed soon after cerebral ischaemia in both the core and penumbral regions.

EICOSANOIDS
Polyunsaturated fatty acids that have widespread biological activities, such as muscle contraction, platelet aggregation and inflammation. Common examples include arachidonic acid, the leukotrienes and the prostanoids.

COMPLEMENT SYSTEM
A set of plasma proteins that attack extracellular pathogens. The pathogen becomes coated with complement proteins that facilitate pathogen removal by phagocytes.

INTERLEUKINS
A generic term for cytokines originally identified as products of leukocytes.

INTERFERONS
Cytokines that promote resistance to viral replication in cells.

CHEMOKINES
Small, secreted proteins that stimulate the motile behaviour of leukocytes.

CASPASES
Cysteine proteases involved in apoptosis, which cleave at specific aspartate residues.

NFκB
A heterodimeric transcription factor for eukaryotic RNA polymerase II promoters.

MITOGEN-ACTIVATED PROTEIN KINASE CASCADE
A signalling cascade that relays signals from the plasma membrane to the nucleus. MAPKs are activated by a wide range of proliferation- or differentiation-inducing signals.

FRACTALKINE
A membrane-bound chemokine that is highly expressed on activated endothelial cells, and is both an adhesion molecule and an attractant for T cells and monocytes.

RANTES
A chemokine that inhibits the infection of T cells by primary HIV-1 strains. RANTES stands for 'regulated upon activation, normal T-cell expressed, and presumably secreted'.

expressed in the CNS[4]. Similarly, IL-1α and IL-1β (two IL-1 agonists) and IL-1ra (a naturally occurring IL-1 receptor antagonist) are produced as precursors. Pro-IL-1α and pro-IL-1ra are active, but pro-IL-1β is inactive and must be cleaved by an IL-1 converting enzyme (ICE, also known as CASPASE 1)[5], which is also required for cellular release of the mature IL-1. The biology of IL-1 has recently become even more complex with the discovery of new putative IL-1 ligands that share sequence homology with IL-1α, IL-1β and IL-1ra[6,7]. Their biological functions are unknown and, although some recent functional data have been described[8], no data are so far available about their role in the CNS.

The TGFβ family includes many proteins (TGFβ1–3, activin, inhibin and several others), although studies on acute neurodegeneration have focused mainly on TGFβ1 (REFS 9,10). Several TGFβ isoforms are expressed by glia and neurons, and their secretion and activation are regulated by latency-associated proteins (LAPs) and by latent TGFβ-binding proteins (LTBPs)[11].

Receptors, signalling pathways and the regulation of cytokine bioactivity are extremely complex. TNFα acts on two high-affinity receptors — TNFR1 (p55) and TNFR2 (p75). Both receptors are expressed in the brain and share some signalling pathways with IL-1RI, the primary IL-1 receptor. These signalling pathways include NFκB and MITOGEN-ACTIVATED PROTEIN KINASES (MAPKs). Receptors for TGFβ (TβR-I and TβR-II) have also been identified in the brain[12,13]. Many other cytokines and their receptors have been identified in the CNS, such as IL-2, IL-3, IL-10, chemokines (for example, FRACTALKINE, IL-8 and RANTES) and the NEUROPOIETIC CYTOKINES (for example, IL-6, IL-11 and leukaemia inhibitory factor).

Complex interactions have been described for cytokine actions in the periphery, including overlapping, synergistic and antagonistic activities. Some data indicate that similar interactions might contribute to neurodegeneration (see below). The concept of distinct groups of 'pro-inflammatory' (for example, IL-1, TNFα and IL-6) and 'anti-inflammatory' (for example, IL-1ra, IL-10 and TGFβ) cytokines has emerged partly on the basis of their actions in the periphery. However, these concepts do not necessarily translate in a direct manner to neurotoxic or neuroprotective actions in the CNS.

Contribution of cytokines to neurodegeneration

Our understanding of the contribution of cytokines to neurodegeneration relies on indirect evidence, which is based largely on changes in expression, and effects of recombinant cytokines, and on more direct studies in which the endogenous cytokine has been modified.

Indirect evidence. The expression of both pro-inflammatory and anti-inflammatory cytokines is induced rapidly by experimental focal or global ischaemia, neonatal hypoxic injury, excitotoxicity, brain trauma and in response to epileptogenic agents in rodents[2,3,10]. The temporal profile of expression depends on the stimulus. Increases in TNFα and IL-1 have been observed before significant neuronal death, as early as 1 h after the insult[14–16], whereas others are expressed slightly later, up to 24 h afterwards (FIG. 2). Many clinical studies have also reported increased expression of cytokines in the cerebrospinal fluid or in post-mortem brain tissue of patients that had suffered stroke or brain injury. In several cases (for example, in the case of IL-6 and TNFα), the levels of expression correlate with the extent of tissue injury and/or with the clinical outcome[17,18]. However, the time course and cellular location of cytokine expression have not been reported in most clinical studies, and it is difficult to distinguish between the cytokine response to injury and the early expression that might contribute to cell death.

When administered individually, cytokines tend not to evoke cell death directly. But when co-administered (for example, IL-1 plus TNFα or IFNγ), they can have synergistic effects that result in neurotoxicity[19,20]. However, recent *in vitro* or *ex vivo* studies indicate that some cytokines do directly induce cell death. For example, exogenous TNFα results in dose-dependent cytotoxicity in primary septo-hippocampal cultures[21]. TNFα also induces apoptotic cell death, but inhibits necrosis, in primary cortical neurons, and evokes both apoptotic and necrotic death in developing PC12 cells[22]. In addition, the intracerebral injection of recombinant TNFα or IL-1 markedly exacerbates ischaemic and excitotoxic injury *in vivo*[23–26], whereas administration of TGFβ, IL-10 or IL-1ra reduces injury[27–31]. Mice that overexpress TNFα in astrocytes, but not in neurons, develop neurological disease[32]. By contrast, several *in vitro* studies report neuroprotective actions of TNFα and IL-1 in cultured neurons or brain slices[33–35], although neurotoxic effects have been seen *in vitro*, usually at very high doses[34]. These conflicting data might reflect indirect actions of cytokines on a

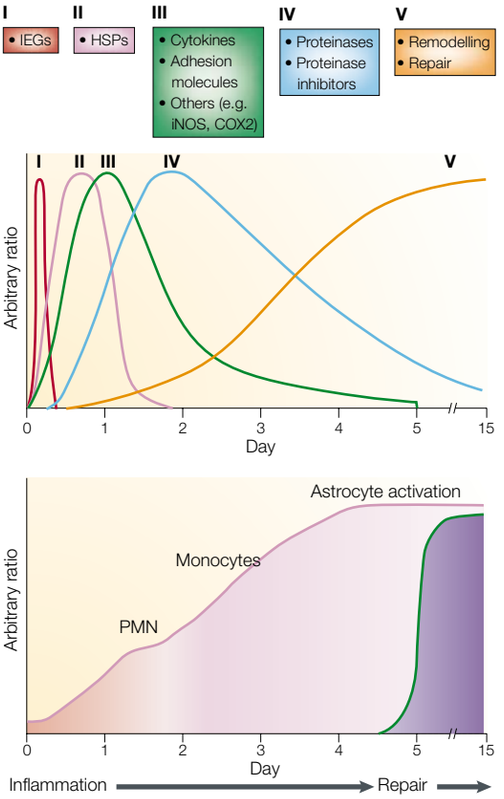


Figure 2 | **Cytokine expression profile.** The time course of expression of putative mediators and modulators of ischaemic brain damage on the basis of gene expression profiles in rodents (upper panel). Phase I: IMMEDIATE-EARLY GENE (IEG) expression. Phase II: HEAT-SHOCK PROTEINS (HSP). Phase III: cytokines and adhesion molecules. Phase IV: proteinase and proteinase inhibitor expression. Phase V: delayed expression of proteins involved in remodelling and repair. The lower panel depicts the time course of cellular events that occur in response to cerebral ischaemia. COX2, cyclooxygenase 2; iNOS, inducible nitric oxide synthase; PMN, polymorphonuclear leukocytes. Figure courtesy of Giora Feuerstein, DuPont Pharmaceuticals Co., Delaware, USA.

NEUROPOIETIC CYTOKINES
Cytokines that regulate cell number in the nervous system and might influence neuronal properties, such as the type of neurotransmitter used by certain neurons. They include ciliary neurotrophic factor and leukaemia-inhibitory factor.

IMMEDIATE-EARLY GENES
Genes that are expressed as one of the earliest responses of cells to factors that initiate the transition between the quiescent and activated states.

HEAT-SHOCK PROTEINS
Molecues that are synthesized in response to increased temperature. They function mainly as chaperones, protecting proteins as they become unfolded due to heating and enabling them to refold correctly.

EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS
A rodent model of multiple sclerosis that is characterized by episodes of spasticity and tremor.

NEUROPROTECTIVE PRECONDITIONING
A phenomenon whereby a brief interruption of blood supply to the brain protects the tissue from a subsequent ischaemic episode.

SPREADING DEPRESSION
A slowly moving depression of electrical activity in the cerebral cortex. It consists of a wave of depolarization that can last for up to two minutes and travels at a speed between three and 12 millimeters per minute. Wave passage is accompanied by increased blood flow, and is followed by a prolonged period of vasodilation. Spreading depression seems to be related to migraine, and has been observed to accompany cerebral ischaemia.

variety of cell types in the CNS, and might also be related to the dose and insult used.

In general, classical pro-inflammatory cytokines (TNFα and IL-1) seem to be neurotoxic, whereas the anti-inflammatory molecules (IL-10 and IL-1ra) are neuroprotective. However, IL-6, a classical pro-inflammatory cytokine that shares many actions with IL-1, inhibits cell death when administered intracerebrally to rodents that are exposed to ischaemic or excitotoxic insults[36,37]. By contrast, mice that overexpress IL-6 show marked neurodegeneration[38], which indicates that chronic IL-6 expression has neurotoxic effects.

Direct evidence. The actions of exogenous cytokines cannot be extrapolated readily to actions of the endogenous protein, which is often expressed at low levels by specific cells. Cytokine production might also reflect a response to, rather than active involvement in, neurodegeneration. More direct evidence for the contribution of cytokines to neurodegeneration derives from studies in which the expression, release or biological activity of the endogenous molecule has been modified.

Acute inhibition of endogenous TNFα by treatment with soluble TNF receptor (which blocks the activity of TNFα), neutralizing antibodies or antisense oligonucleotides markedly reduces ischaemic or traumatic brain damage in rodents[26,39,40]. These findings indicate that endogenous TNFα contributes directly to neuronal injury, but other studies indicate a neuroprotective role of endogenous TNFα. Mice lacking either the p55 receptor, or both the p55 and p75 TNF receptors, show enhanced ischaemic and excitotoxic injury compared with wild-type or p75-receptor-deficient animals[35,41]. By contrast, mice that lack TNFα show improved initial recovery in response to traumatic brain injury (1–2 days after the insult), as compared with wild-type mice. However, TNFα-deficient mice show greater neurological dysfunction at later times (2–4 weeks)[42]. So, TNFα might contribute to early neuronal injury, but could improve recovery. However, these apparent neuroprotective actions of endogenous TNFα are modest compared with the effect of neurotrophins or growth factors. In addition, studies on knockout mice might be somewhat misleading because of developmental changes and/or deletion of both the peripheral and central actions of TNFα[43].

Many studies have indicated that endogenous IL-1 directly contributes to experimentally induced neurodegeneration. The administration of recombinant IL-1ra[44] into the brain or periphery of rodents markedly inhibits brain damage that is caused by cerebral ischaemia, brain injury or excitotoxins[27,45,46]. Similarly, overexpression of IL-1ra in the brain also inhibits ischaemic brain damage[47]. Injection of an anti-IL-1β antibody is also neuroprotective[23], indicating that IL-1β is the main ligand that mediates the toxic effect. However, deletion of IL-1α or IL-1β alone in mice fails to modify damage caused by middle cerebral artery occlusion (MCAo), whereas deletion of both ligands markedly reduces damage[48]. Inhibition of the actions of endogenous IL-1ra by administration of a neutralizing antibody increases ischaemic brain damage in the rat[49], which indicates that IL-1ra is a functional endogenous inhibitor of neuronal damage.

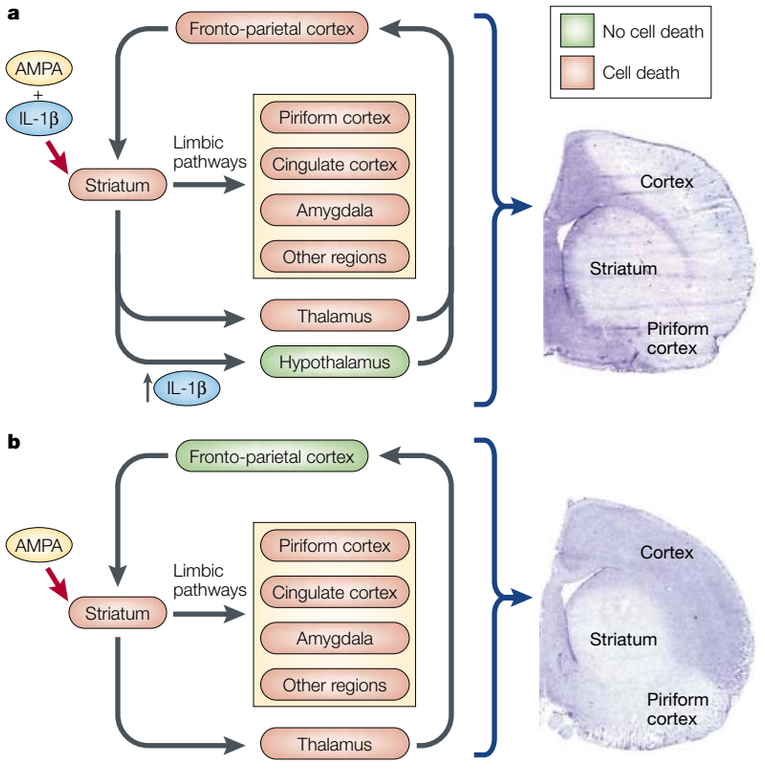
Intracerebral injection of a soluble TGFβ type II receptor, which binds and inactivates TGFβ, exacerbates both ischaemic and excitotoxic lesions in the rat brain[50], further supporting a role of TGFβ as a neuroprotective cytokine. Although the effects of chronic deletion of TGFβ in neurodegeneration have not been studied, mice that overexpress TGFβ show enhanced susceptibility to EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS, a CNS disease that is mediated by the immune system[51].

Studies in mice in which the IL-6 gene has been deleted have provided conflicting data. One study reports a reduced inflammatory response and increased neuronal death after cryo-injury[52], whereas another found no difference between IL-6 knockout and wild-type animals in their response to ischaemic injury[53]. The absence of inhibitors that are selective for IL-6 has hampered progress in defining whether endogenous IL-6 does indeed exert neuroprotective effects.

Box 1 | **Interleukin 1 and seizures**

Epileptic activity after cerebral ischaemia or head injury might contribute to secondary brain damage and functional outcome. Inflammatory cytokines, particularly interleukin 1 (IL-1), have been proposed to participate in the pathogenesis of seizures. This proposal is based on different lines of evidence. For example, seizures stimulate IL-1α, IL-1β, IL-6, IL-1ra and TNFα levels in the brain[150]. Furthermore, intracerebral IL-1 administration enhances kainate-induced seizures[151], central application of IL-1ra is anticonvulsant[152], IL-1 levels are increased during epilepsy in the human brain[153], and IL-1β gene polymorphisms have been associated with temporal lobe epilepsy[154].

The mechanisms by which IL-1 affects seizure activity are not known, but we have shown that IL-1 administered with the excitotoxin AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid) in the rat striatum produces widespread cortical cell death (**a**) that is not observed with either AMPA (**b**) or IL-1 alone (paler areas in the histological sections). Furthermore, this cell death is mediated by actions in the hypothalamus, together with the activation of neuronal pathways that are associated with seizures and the limbic system[155]. Seizures elicited by kainate injections in discrete brain regions propagate to and produce cell death in distant brain areas that are connected by neuronal projections[156]. IL-1 might therefore propagate signals through the brain to act in specific brain regions, and exert its effect on seizure activity and neurodegeneration[157]



Overall, the data indicate that IL-1 contributes to neurodegeneration, whereas TGFβ is neuroprotective and IL-10 inhibits injury[30,31]. However, studies on TNFα and IL-6 indicate that they might both contribute to and inhibit neuronal death. These apparent discrepancies might be related to the expression timing and site, and to their concentration. So, for example, IL-1 and TNFα, which clearly exacerbate ischaemic or excitotoxic brain injury when administered at the time of CNS insults, are neuroprotective when administered before the insult, and are believed to contribute to the phenomenon of NEUROPROTECTIVE PRECONDITIONING[54].

Regulation of cytokine bioactivity

Cytokine bioactivity can be regulated at the levels of transcription, translation, cleavage and cellular release, as well as through receptor and post-receptor signalling mechanisms. The activity of many cytokines (particularly IL-1 and TNFα) is also modifiable by soluble binding proteins or receptors, and, in the case of IL-1, by the endogenous receptor antagonist IL-1ra.

It seems that all cell types (neurons, glia and endothelial cells) in the CNS can express TNFα. The bioactivity of TNFα is influenced by endogenous TNFα-binding protein (TBP), a soluble fragment of the TNFα receptor that reduces neuronal loss after head injury in rodents[55]. TNFα can also induce IL-10, which itself inhibits the expression of TNFα, resulting in an autoregulatory feedback loop[56]. TNFα release from microglia is also inhibited by the chemokine fractalkine, which is expressed predominantly in the CNS[57].

Among the different IL-1 family members, IL-1β is the most rapidly expressed in response to brain damage, initially by microglia[58]. The mechanisms of cellular release of IL-1, which lacks a classical leader sequence, are largely unknown, but seem to be linked closely to cleavage by caspase 1 and depend on activation of purinergic P2X7 receptors in macrophages and microglia[59]. Extracellular ATP, which is released from damaged cells, activates the P2X7 receptor, leading to reduced intracellular K^+ levels, activation of caspase 1, IL-1β release and associated macrophage or microglial cell death[60]. However, IL-1β release seems to be independent of this death[59]. Increased expression of P2X7 receptors occurs within an ischaemic infarct[61], although a key question is whether sufficient ATP is available under conditions of ischaemia.

As discussed above, the mechanisms of TGFβ regulation are complex, and little is known about these events in the CNS, although upregulation of LTBP2 production by TGFβ has been described in astrocytes, which could lead to self-activation[62]. TGFβ isoforms have distinct promoter regions that regulate their transcription and translation, leading to differential expression in response to a particular stimulus. This could serve as a means of regulating their activity[10].

The mechanisms that regulate the expression and release of these (and other) cytokines are closely related. So, TNFα induces the expression of IL-6 and TGFβ[63,64]. Similarly, IL-1 can induce IL-6, TNFα and TGFβ expression[63,65,66], whereas TGFβ suppresses TNFα production[67]. The primary stimuli for cytokine expression in the CNS are largely unknown, particularly as their induction seems to occur before detectable cell death. An early and direct stimulus to cytokine expression is excessive neuronal activity, which can be manifested as local depolarization, SPREADING DEPRESSION[68] or seizures (BOX 1), and can occur within seconds of a CNS insult. It remains unclear what level of neuronal activity induces cytokines (for example, whether they can be induced by 'physiological' overactivation), what their role in physiological neuronal processes is, and whether neuronal activity is the primary stimulus for cytokine expression.

Several factors are known to modulate cytokine expression, some of which influence induction in the CNS and might in turn modify neurodegeneration. These stimuli include physical or psychological stress, and infection or inflammation within the brain or the periphery[69]. The latter, probably mediated in part through vagal activation[70,71], could explain the reported association between systemic infection or inflammation, and poor outcome after stroke or head injury, although this might also be related to factors such as fever, which exacerbates neuronal injury[72]. Excitatory amino acids might regulate cytokine expression directly, particularly during excessive release after CNS injury. Recent data indicate that the postsynaptic density protein 95 (PSD95) binds the activated NMDA (*N*-methyl-D-aspartate) receptor subunit NR2 and the kainate receptor subunit GluR6, binding that in turn triggers phosphorylation of C-JUN N-TERMINAL KINASE (JNK)[73,74]. JNK activates the transcriptional factor JUN, which promotes the expression of cytokines involved in inflammation, such as IL-1, IL-6, TNFα and IFNα/γ. Anti-inflammatory agents such as GLUCOCORTICOIDS are potent inhibitors of IL-1 and TNFα expression[75], yet their effects on neuronal survival are complex[76]. Similarly, CANNABINOIDS inhibit IL-1 and TNFα expression and release from glia[77], and have anti-inflammatory and neuroprotective actions *in vitro* and *in vivo*[78].

Chemokines are upregulated in response to ischaemia and CNS trauma — initially in astrocytes, and subsequently in infiltrating macrophages and reactive microglia within the damaged tissue — and regulate the movement of inflammatory cells into the CNS[79], which is a crucial step in response to traumatic and ischaemic insults. Constitutively expressed fractalkine tonically regulates TNFα expression in the CNS, and the neutralization of endogenous fractalkine with a specific antibody potentiates LIPOPOLYSACCHARIDE-stimulated release of TNFα in the brain[80]. Cytokines can modify the expression of chemokines and their receptors, TGFβ selectively increases chemokine receptor 1 in astrocytes[81], IL-1 and TNFα stimulate RANTES production by both microglia and astrocytes, and TGFβ and IL-10 downregulate the production of this and other chemokines[82,83].

Mechanisms of action

In view of the diversity of cytokine actions, it is no surprise that their specific sites and mechanisms of action in neurodegeneration have proved to be difficult to unravel. Cytokines can exert direct actions on neurons (usually shown *in vitro*) and indirect actions on glia, on the brain vasculature, and on physiological parameters such as regional blood flow or temperature. Many, if not all, of these effects probably influence functional outcomes related to neurodegeneration.

Common pathways of neuronal cell death have been identified in response to diverse insults, such as ischaemia, trauma or excitotoxicity. These include early disruption of ion homeostasis, excessive neuronal activation, seizures and spreading depression, massive release and impaired uptake of neurotransmitters such as glutamate, intracellular entry of Ca^{2+}, and release of nitric oxide and free radicals. More recently, further factors have been identified, including activation of genes that initiate or execute apoptosis, and the influence of glial and endothelial cells, extracellular matrix and invading immune cells. There is evidence that specific cytokines can act at most, if not all, of these steps, and probably have multiple actions on several cells or systems involved in neurodegeneration.

Cytokine receptors and signalling. The constitutive expression of cytokine receptors (for example, receptors for TNFα, IL-1, TGFβ, IL-2, IL-6, IL-8, IL-10 and IL-11), albeit at low levels, has been reported on most cell types throughout the brain, and these often show rapid upregulation in response to injury.

IL-1 and TNFα act on distinct cell-surface receptors, but share some common signalling mechanisms (FIG. 3), some of which have been identified in the CNS and relate to neurodegeneration. Increased expression of p38 MAPK and extracellular-signal-regulated kinase (ERK) has been found in ischaemic brain tissue after MCAo[84]. Selective inhibitors of these pathways markedly reduce the ischaemic injury in rodents[85,86].

TNFR1 and TNFR2 belong to the low-affinity neurotrophin receptor gene superfamily. TNFα elicits its biological effects on multiple cell types in the CNS through these receptors[87]. IL-1 receptor expression has been described on both glia and neurons in several brain regions[88], but the distribution of IL-1RI in brain does not correlate closely with the identified sites of IL-1 action in the striatum and hypothalamus[89]. IL-1 is believed to signal through a single receptor (IL-1RI) that requires association with an accessory protein[90], and binds IL-1α and IL-1β with equal affinity. However, several pieces of indirect evidence question this view[89] and indicate that IL-1 might signal through other receptors in the CNS. IL-1Rs form part of the large Toll receptor superfamily, identified initially in *Drosophila*[91]. The role of new IL-1-like receptors in the CNS is unclear, but one of them — Toll-like receptor 4 (TLR4) — has been reported in brain, mainly in the CIRCUMVENTRICULAR ORGANS[92], and another one — IL-1RAPL — is expressed in the hippocampus. Mutations of IL-1RAPL are associated with X-linked mental retardation[93].

TGFβ exerts its biological actions through a heteromeric complex of two receptor subunits, TβR-I and TβR-II[94]. TβR-II, which is constitutively phosphorylated, binds TGFβ, leading to recruitment and transphosphorylation of TβR-I, which then signals through activation of the SMAD transcription-factor cascade[95]. TGFβ-mediated signalling is also regulated through crosstalk with other signal transduction pathways, including MAPK. This observation might explain interactions between TGFβ and pro-inflammatory cytokines.

Receptors for several other cytokines have been identified in the CNS. IL-6 acts through binding to its specific receptor subunit (IL-6R) and the signal transducer gp130, which are both distributed widely throughout the brain on both glial and neuronal

PSD95
A protein of the postsynaptic density, which can interact with NMDA receptors. It is thought to participate in regulating the spatial distribution of this receptor subtype.

C-JUN N-TERMINAL KINASES
A family of kinases distantly related to extracellular-signal-regulated kinases (ERKs) that are activated by dual phosphorylation on tyrosine and threonine residues.

GLUCOCORTICOIDS
Hormones produced by the adrenal cortex, which are involved in carbohydrate and protein metabolism, but also affect brain function. Cortisol (human) and corticosterone (rodent) are prime examples.

CANNABINOIDS
Derivatives of 2-(2,2-isopropyl-5-methylphenyl)-5-pentyl-resorcinol, a molecule found in the plant *Cannabis sativa*. Cannabinoids are responsible for the psychoactive effects of marijuana.

LIPOPOLYSACCHARIDE
A toxic component of the outer cell wall of gram-negative bacteria.

CIRCUMVENTRICULAR ORGANS
Some of the structures located around the wall of the ventricular system, which are characterized by the absence of blood–brain barrier.

SMADS
A family of transcription factors that mediate TGFβ signals. The term SMAD is derived from the founding members of this family, the *Drosophila* protein MAD (mothers against decapentaplegic) and the *Caenorhabditis elegans* protein SMA (small body size).

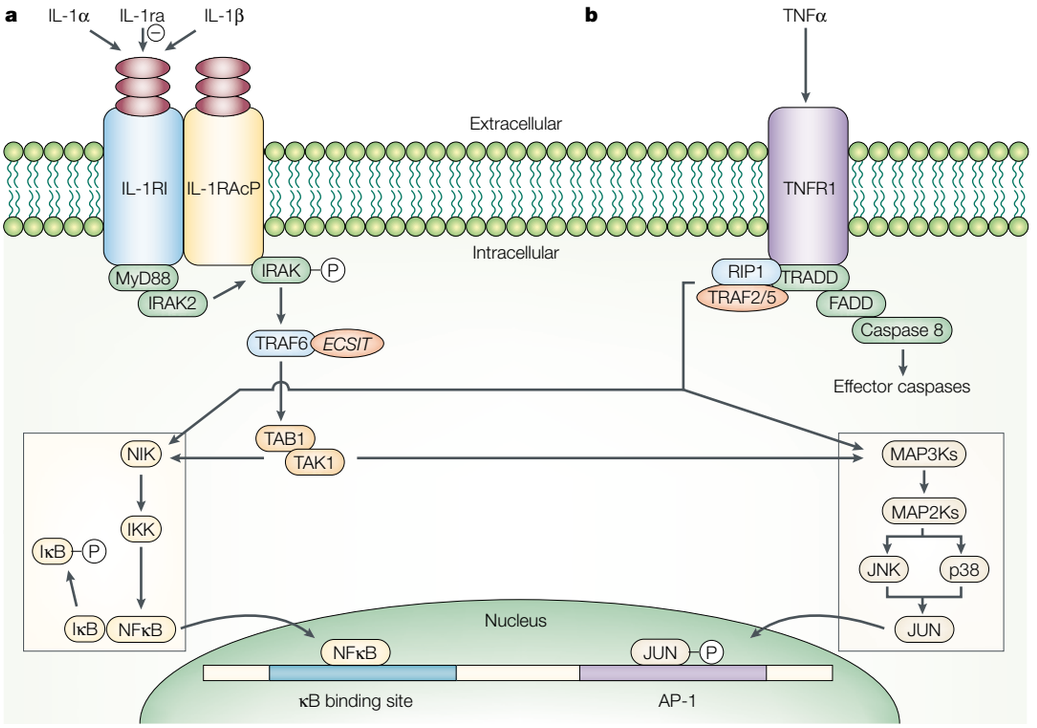


Figure 3 | **Signal transduction through IL-1RI and TNFR1. a** | The two interleukin 1 agonists (IL-1α and IL-1β) signal through a membrane-associated receptor (IL-1RI), which acts in concert with the IL-1 receptor accessory protein (IL-1RAcP) to initiate signal transduction. IL-1ra prevents the association of IL-1RI and IL-1RAcP. Formation of the IL-1RI/IL-1RAcP complex, together with the adaptor molecule MyD88, leads to recruitment of the IL-1 receptor kinases IRAK and IRAK2, which subsequently recruit tumour necrosis factor receptor (TNFR)-associated factor 6 (TRAF6). This leads to the activation of NFκB and mitogen-activated protein kinase (MAPK) signalling pathways through transforming growth factor-β (TGFβ)-activated kinase 1 (TAK1) and TAK1-binding protein (TAB1). This chain of events results in the activation of nuclear transcription factors that subsequently induce the expression of many genes. **b** | The biological activity of tumour necrosis factor-α (TNFα) is mediated by TNF receptors 1 (TNFR1) and 2, which form trimers when bound by the ligand. Activation of TNFR1 leads to recruitment of TRAF2/5, RIP1 and FADD, through the adaptor protein TRADD. The subsequent recruitment and activation of caspase 8 by FADD can lead to apoptosis. TRAF2/5 and RIP1 lead to the activation of NFκB and AP-1 through the activation of IKK and MAPKs. So, TNFα shares several downstream signalling pathways with IL-1. It is important to note that this schematic representation does not include all information available about these pathways, but serves as a simple overview. AP-1, adaptor protein complex 1; FADD, Fas-associated protein with death domain; IKK, inducible IκB kinase; JNK, c-Jun N-terminal kinase; MAP2Ks, MAPK kinase; MAP3Ks, MAPK kinase kinase; MyD88, myeloid differentiation primary response gene, NIK, NFκB-inducing kinase; p38, p38 MAPK; RIP1, receptor-interacting protein 1; TRADD, TNFR1-associated protein with death domain.

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cells[96]. Several other cytokines, including leukaemia inhibitory factor, IL-11 and ciliary neurotrophic factor, also signal through gp130 (REF. 97). Little is known about the expression and signalling mechanisms of IL-10 in the brain, although its receptor is present on both microglia and astrocytes[98], and activation of IL-10 receptors stimulates STAT type 3, which might represent the pathway by which IL-10 inhibits the apoptotic death of microglia[99].

The cytokines described above have distinct receptors, but share many common signalling mechanisms, providing potential for interactions at this level. Venters and colleagues[100] showed that TNFα fails to elicit cell death directly, but acts through an interaction with the insulin-like growth factor 1 signalling pathway, a factor that has neuroprotective actions *in vivo* and *in vitro*. This 'silencing of survival signals' might have relevance to the actions of other cytokines in mediating cell death.

Cytokine actions on neurons. Direct actions of cytokines on neuronal functions (for example, transmitter release and ion channel activity) can contribute to neuronal injury (see below). This evidence is largely derived from studies of the effects of recombinant cytokines *in vitro*, and, in some cases, the results conflict with the *in vivo* data. Cell-culture systems usually comprise immature cells and lack glia or synaptic connections that might be essential for cytokine action. Although *ex vivo* brain slices retain some of the brain architecture and circuitry, they are often injured during preparation, resulting in an excessive release of toxins and inflammatory mediators that can compromise the results.

In vivo IL-1 and TNFα acutely enhance neuronal injury, yet TNFα induces the expression of the BCL PROTEINS BCL2 and BCLX in hippocampal neurons *in vitro* through NFκB activation, which protects against hypoxic injury[101]. Several reported effects of IL-1 on

STATS
A family of cytoplasmic transcription factors (signal transducers and activators of transcription) that dimerize on phosphorylation and translocate to the nucleus to activate the transcription of target genes.

BCL PROTEINS
Molecules that are associated with B-cell leukaemia and lymphoma. BCL2 is a mitochondrial protein of the inner membrane, which can block apoptosis. BCLX is also a regulator of apoptosis, which exists in two forms: long and short.

neurons could reduce neuronal death; for example, inhibition of Ca^{2+} entry into neurons[102], inhibition of glutamate release[103], inhibition of LONG-TERM POTENTIATION[104] and enhanced GABA (γ-aminobutyric acid)-mediated activity[105]. However, other actions of IL-1, which could depend on its effects on neurons, could contribute to neurodegeneration. These include the induction of cyclooxygenase 2 (COX2) and inducible nitric oxide synthase (iNOS)[106]. IL-1 is also a potent inducer of the hypothalamic neuropeptide corticotrophin releasing factor (CRF), which has been implicated in ischaemic and traumatic brain injury[107,108], through mechanisms that remain unknown. TGFβ directly inhibits the rise in neuronal intracellular Ca^{2+} levels and subsequent neurotoxicity produced by NMDA[109], and increased expression of COX2 is seen in neurons in response to TGFβ[110].

Cytokine actions on glia. Neuronal survival is critically dependent on glial function, which can exert both neuroprotective and neurotoxic influences[111]. Glial cells are a primary target of cytokines and are activated in response to many cytokines, including TNFα and IL-1. This activation can trigger further release of cytokines that might enhance or suppress local inflammatory responses and neuronal survival. Glia, particularly astrocytes, are a principal source of neurotrophins and growth factors, such as nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF) and glial-derived neurotrophic factor (GDNF), which are induced by cytokines and exert neuroprotective actions. By contrast, all glial subtypes, particularly microglia, can express potentially neurotoxic factors[112].

TNFα directly affects astrocytes, inducing a slow increase in intracellular Ca^{2+} and marked depolarization, and reducing glutamate-evoked rises in Ca^{2+} (REF. 113), which would affect synaptic transmission indirectly. The contribution of IL-1 to neurodegeneration might depend on the balance between neuroprotective and neurotoxic factors released from glia. IL-1 induces NGF expression and release in astrocytes[114], which exerts neuroprotective actions *in vitro*[34]. Giulian *et al.* suggest that IL-1 releases neurotoxins from glia[111], and recent data indicate that conditioned medium from IL-1-treated glia is neurotoxic to cortical neurons (M. W. Craighead and N. J. R., unpublished data). The specific nature of these neurotoxins has not been fully elucidated, although there are several candidates that include nitric oxide[115], quinolinic acid[116], acute-phase proteins (for example, β-amyloid precursor protein and α1-antichymotrypsin)[117] and complement[118].

The protective effects of TGFβ against NMDA-induced necrosis depend on the upregulation of plasminogen activator inhibitor 1 (PAI1) in astrocytes[119]. TGFβ might indirectly affect neuronal survival through its interaction with NGF and other neurotrophic factors[120,121]. In microglia, TGFβ selectively elicits apoptosis through a BCL2-independent mechanism[122] that might suppress the detrimental effects of activated microglia on neighbouring cells[123].

Cytokines and apoptosis. Cytokines directly influence apoptosis in non-neuronal cells (for example, in cells of the immune system), but the contribution of apoptosis to neuronal death in the adult CNS remains controversial, and depends in part on both the experimental system, and the definition and criteria for apoptotic cell death. There is relatively little morphological evidence for apoptotic cell death in the adult CNS, although the contribution of apoptotic mechanisms to neurodegeneration is now well established. Several caspases and other genes involved in apoptosis are activated in the CNS of experimental animals in response to diverse insults. Genetic deletion of caspase 1 (REF. 124), caspase 11 (REF. 125) or p53 (REF. 126), and administration of caspase inhibitors[127], reduce ischaemic damage in rodents, although no selective inhibitors have been tested as yet.

TNFα can induce apoptosis directly, through the activation of FAS receptors that are known to be present in the CNS[128], and can also activate signalling mechanisms that are involved in apoptosis (for example, Fas-associated protein with death domain, FADD). The involvement of IL-1 in apoptosis is less clear. IL-1 alone does not induce apoptosis in healthy, proliferating cells, but was shown to evoke apoptotic cell death directly in non-dividing HeLa cells[129]. These observations have not been repeated in neurons, although IL-1 applied with IFNγ can cause apoptosis in primary cultures of human fetal neurons[20]. Caspase 1, the enzyme required to cleave pro-IL-1β and pro-IL-18 to their active forms, might contribute to apoptosis as an 'activator' caspase[130].

TGFβ induces BCL2 and BCLX[131], and inhibits caspase 3 (REF. 132), which reduces apoptosis in hippocampal neurons. TGFβ also elicits the expression of FADD-like ICE-inhibitory protein (FLIP) in resting and activated microglia, through a MAPK kinase (MKK)-dependent pathway[133]. FLIP can interfere with the FasL-induced activation of caspase 8 and caspase 3. So, the upregulation by TGFβ prevents subsequent cell death.

Other actions of cytokines. Neurodegeneration and clinical outcomes are initially dependent on disruption to or activation of systems within and outside the CNS, which might be influenced by cytokines. Ischaemia is the primary cause of neuronal injury in stroke and is a main contributor to secondary damage after brain trauma. The limited reports available so far indicate that cytokines do not influence cerebral blood flow directly, but they do have numerous actions on the vasculature that might influence neuronal survival, and IL-1 induces neovascularization in the CNS[134]. Pro-inflammatory cytokines, such as IL-1 and TNFα, can cause damage to the blood–brain barrier[135], release neurotoxins such as nitric oxide from the vascular endothelium[136], cause the upregulation of adhesion molecules involved in the invasion of leukocytes (for example, intercellular cell-adhesion molecule 1)[137] and induce VASOGENIC OEDEMA[138].

Pro-inflammatory cytokines, such as IL-1, IL-6 and TNFα, are key mediators of host defence responses, such as fever, immune activation, endocrine, metabolic and cardiovascular changes[139]. Many of these responses are

LONG-TERM POTENTIATION
A long-lasting increase in the efficacy of synaptic transmission, commonly elicited by high-frequency neuron stimulation.

FAS
A transmembrane protein that mediates apoptosis and might be involved in the negative selection of autoreactive T cells in the thymus.

VASOGENIC OEDEMA
The accumulation of extracellular fluid that results from changes in capillary permeability, allowing for the seepage of plasma molecules and water.

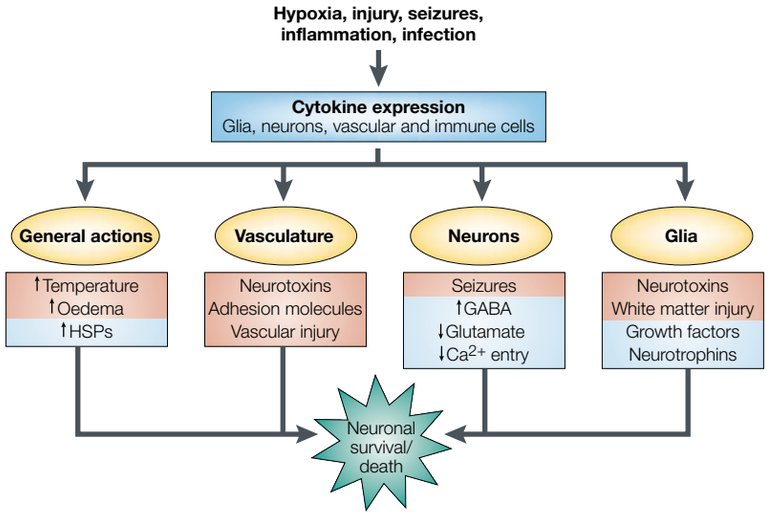

Figure 4 | **Summary of putative actions of cytokines in neurodegeneration.** Cytokines can be expressed by numerous cell types early after brain insults. They can then have actions on many aspects of central nervous system function that might contribute to or limit subsequent neuronal injury. HSP, heat-shock protein. Red boxes, factors that promote cell death; blue boxes, factors that promote cell survival.

mediated or modulated by cytokine actions in the CNS, are induced by acute CNS injury, and might be detrimental to clinical outcome. Hyperthermia markedly exacerbates neuronal injury in experimentally induced cerebral ischaemia or brain trauma[72], and is associated with poor outcome in stroke patients[140]. IL-1, IL-6 and TNFα are important endogenous PYROGENS that act primarily in the hypothalamus to elicit fever through the release of prostaglandins[141]. However, fever is probably not the primary mechanism of action of these cytokines, as cyclooxygenase inhibitors have only modest neuroprotective actions[142]. Other aspects of the acute-phase response induced by cytokines include activation of the hypothalamus–pituitary–adrenal axis, redirection of blood flow, increased circulating acute-phase proteins and leukocytes, all of which could influence the outcome after stroke or head injury. All of these effects are likely to exacerbate neuronal loss, but could be secondary to the primary sites of cytokine action in neurodegeneration.

In the periphery, the synthesis and bioavailability of cytokines can be infuenced by the extracellular matrix[143,144]. The role of extracellular matrix in the injured CNS has begun to emerge only recently with reports of deposition of proteins such as fibronectin[145]. Notably, deletion of plasma fibronectin in mice markedly exacerbates ischaemic brain damage[146].

Summary and future research

Numerous cytokines are induced rapidly after acute CNS insults, and are expressed in a temporal and spatial pattern consistent with their involvement in subsequent neuronal death. Modulation of exogenous or endogenous cytokines *in vivo* and *in vitro* has yielded conflicting data. Overall, IL-1 seems to contribute directly to neurodegeneration, whereas IL-1ra, IL-10 and TGFβ are neuroprotective. Finally, IL-6 and TNFα can both enhance and inhibit neuronal injury, probably depending on the time course and extent of expression.

The complex actions and putative mechanisms of cytokines in the CNS are similar to their functions in the periphery. These molecules can act at very low concentrations on numerous cell types within or outside the CNS. It is likely that the contribution of cytokines to neurodegeneration does not involve a single mechanism on one specific cell type, but rather depends on several actions, which might be detrimental or beneficial (FIG. 4).

It is now important to identify the primary mechanisms that are involved in regulating cytokine bioavailability, and the specific sites and mechanisms of action that result in neuronal death or survival; this information could allow more effective therapeutic intervention.

Clinical relevance and therapeutic targets

Clinical studies on the modification of cytokines in CNS disease are limited. IFNβ is widely used in the treatment of MULTIPLE SCLEROSIS, although its mechanism of action is still largely unknown[147]. Several growth factors (GDNF, BDNF and NGF) have been tested in acute or chronic neurodegenerative diseases, but without significant efficacy.

Experimental studies indicate that TGFβ, IL-6 or IL-10 could be of therapeutic benefit in protecting neurons, but given their pleiotropic actions (including some inflammatory effects of TGFβ and IL-6), all might give rise to unwanted side effects. Acute inhibition of TNFα (for example, with anti-TNFα antibody or soluble receptor) has been shown to have some efficacy in experimental stroke, but the potential protective effects of endogenous TNFα call this strategy into question. The picture is somewhat clearer for IL-1, as modification of its release or actions markedly reduces neuronal injury, oedema and glial activation caused by cerebral ischaemia or injury in rodents, and IL-1ra improves behavioural outcome.

Several targets have been identified for the modification of IL-1 bioactivity, including inhibition of microglial activation, the function of P2X7 receptors, caspase 1 activity, receptor binding and intracellular signalling pathways. The most advanced and intensely studied is IL-1 receptor blockade with recombinant IL-1ra. This protein has been tested in normal volunteers, subacutely in SEPSIS and chronically in rheumatoid arthritis, with few or no side effects and, in the latter condition, with significant efficacy[148].

In mice, peripherally administered IL-1ra enters the brain, albeit at a low level[149], and is neuroprotective in several forms of CNS injury in models. So, IL-1ra, or a small molecule antagonist of IL-1 receptors, might be beneficial in acute neurodegenerative conditions.

So far, clinical trials of all other neuroprotective agents have failed in these conditions. This might be due, at least in part, to dose-limiting side effects. Inhibiting IL-1 has no reported side effects in animals or humans, and no significant physiological role of IL-1 has been identified. So, the main challenge is probably to deliver sufficient levels of cytokines or their modifiers to the ischaemic or injured brain.

PYROGEN
Any agent capable of producing fever.

MULTIPLE SCLEROSIS
A neurodegenerative disorder characterized by demyelination of central nervous system tracts. Symptoms depend on the site of demyelination and include sensory loss, weakness in leg muscles, speech difficulties, loss of coordination and dizziness.

SEPSIS
Infection of the soft tissues or blood by pathogens that results in tissue destruction.

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Online links

DATABASES
LocusLink: http://www.ncbi.nlm.nih.gov/LocusLink/
BCL2 | BCLX | caspase 1 | COX2 | FADD | FLIP | gp130 | IKK | IL-1α | IL-1β | IL-1ra | IL-1RAcP | IL-1RAPL | IL-1RI | IL-6 | IL-6R | IL-10 | iNOS | intercellular cell-adhesion molecule 1 | LTBP2 | MyD88 | P2X7 receptor | p53 | PAI1 | RIP1 | TβR-I | TβR-II | TGFβ1 | TLR4 | TNFα | TNFR1 | TNFR2 | TRADD

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