(Peri)vascular production and action of pro-inflammatory cytokines in brain pathology

Jan P. KONSMAN*, Benjamin DRUKARCH† and Anne-Marie VAN DAM†

*Laboratory of Integrative Neurobiology, CNRS FRE 2723/INRA UR 1244/University Bordeaux2, Institut François Magendie, Bordeaux, France, and †Department of Anatomy and Neurosciences, VU University Medical Center, Van der Boechorststraat 7, Amsterdam, The Netherlands

ABSTRACT

In response to tissue injury or infection, the peripheral tissue macrophage induces an inflammatory response through the release of IL-1β (interleukin-1β) and TNFα (tumour necrosis factor α). These cytokines stimulate macrophages and endothelial cells to express chemokines and adhesion molecules that attract leucocytes into the peripheral site of injury or infection. The aims of the present review are to (i) discuss the relevance of brain (peri)vascular cells and compartments to bacterial meningitis, HIV-1-associated dementia, multiple sclerosis, ischaemic and traumatic brain injury, and Alzheimer’s disease, and (ii) to provide an overview of the production and action of pro-inflammatory cytokines by (peri)vascular cells in these pathologies of the CNS (central nervous system). The brain (peri)vascular compartments are highly relevant to pathologies affecting the CNS, as infections are almost exclusively blood-borne. Insults disrupt blood and energy flow to neurons, and active brain-to-blood transport mechanisms, which are the bottleneck in the clearance of unwanted molecules from the brain. Perivascular macrophages are the most reactive cell type and produce IL-1β and TNFα after infection or injury to the CNS. The main cellular target for IL-1β and TNFα produced in the brain (peri)vascular compartment is the endothelium, where these cytokines induce the expression of adhesion molecules and promote leucocyte infiltration. Whether this and other effects of IL-1 and TNF in the brain (peri)vascular compartments are detrimental or beneficial in neuropathology remains to be shown and requires a clear understanding of the role of these cytokines in both damaging and repair processes in the CNS.

INTRODUCTION

The inflammatory response is essential for survival in response to tissue injury or infection, but it also has the capacity to cause considerable bystander damage. Regardless of the initiating event, the peripheral tissue macrophage can induce an inflammatory response through the release of the prototypical pro-inflammatory

Key words: blood–brain barrier, central nervous system, endothelium, inflammation, interleukin-1, macrophage, tumour necrosis factor (TNF).

Abbreviations: Aβ, amyloid-β protein; APP, Aβ precursor protein; CNS, central nervous system; CSF, cerebrospinal fluid; EAE, experimental autoimmune encephalomyelitis; GFAP, glial fibrillary acidic protein; GFP, green fluorescent protein; IL-1, interleukin-1; ICE, IL-1β-converting enzyme; IL-1R, IL-1 receptor; IL-1ra, IL-1R antagonist; ISF, interstitial fluid; IκB, inhibitory-κB; JNK, c-Jun N-terminal kinase; LPS, lipopolysaccharide; LRP, low-density lipoprotein receptor-related protein; MAPK, mitogen-activated protein kinase; MADD, MAPK-activating death domain; MS, multiple sclerosis; NF-κB, nuclear factor κB; NGF, nerve growth factor; SCID, severe combined immunodeficient; SIV, simian immunodeficiency virus; TACE, TNFα-converting enzyme; TLR, Toll-like receptor; TNF, tumour necrosis factor; TNFR, TNF receptor; TRADD, TNFR-associated death domain; TRAF, TNFR-associated factor.

Correspondence: Dr Anne-Marie Van Dam (email amw.vandam@vumc.nl).
cytokines IL-1 (interleukin-1) \(\beta\) and TNF (tumour necrosis factor) \(\alpha\) [1]. These cytokines act on endothelial cells to induce the expression of adhesion molecules that slow down circulating leucocytes and make them adhere to the vascular wall [1]. In parallel, IL-1 and TNF\(\alpha\) also stimulate tissue macrophages and endothelial cells to express chemokines, which attract leucocytes into the site of injury or infection [1]. The critical role of these pro-inflammatory cytokines in leucocyte attraction is illustrated by the observation that the local injection of IL-1\(\beta\) or TNF\(\alpha\) alone is sufficient for neutrophil exudation into the skin [2].

The effector functions of the different types of leucocytes and, in particular, neutrophils include bacterial phagocytosis, production of toxic oxidant metabolites and the release of proteolytic enzymes.

In addition, vascular physiology changes occur during local inflammation. Indeed, the vascular tone of post-capillary venules decreases under the influence of prostaglandins induced by the action of IL-1\(\beta\) and TNF\(\alpha\) [1]. Vasodilation and leakage result, in turn, in plasma protein extravasation and oedema [1]. The extravasation of plasma proteins can be dependent on neutrophil recruitment, mediated by the action of molecules such as histamine and bradykinin, or by neurogenic mediators such as substance P [3]. In addition to their effects on vascular tone, prostaglandins, histamine and bradykinin also mediate inflammatory pain [4,5]. Thus the actions of IL-1\(\beta\) and TNF\(\alpha\) on endothelial cells of peripheral tissues play important roles in mediating the hallmarks of the local inflammatory response: vasodilation, oedema, pain and loss of function.

It is intriguing to see how the vascular actions of IL-1\(\beta\) and TNF\(\alpha\) in the local inflammatory response in peripheral tissues have long been acknowledged, whereas this notion is only starting to emerge in central nervous tissue. This delay in our understanding is due in part to the long-held idea that the blood–brain barrier renders the brain an immune-privileged site and the consequence of a certain neurocentric view in brain pathology.

The aim of the present review is, therefore, to provide an overview of the production of pro-inflammatory cytokines by brain vascular and perivascular cells and their role in different pathologies affecting the CNS (central nervous system). The first part of the review describes the unique features of the brain vascular and perivascular (hereafter (peri)vascular) compartments, such as the blood–brain barrier and the perivascular macrophages. The relevance of changes in the (peri)vascular compartments of neurological diseases, such as bacterial meningitis, MS (multiple sclerosis), HIV-1-associated dementia, ischaemic and traumatic brain injury, and Alzheimer’s disease, in which the vasculature is affected to various degrees will also be discussed. As these unique features of the (peri)vascular compartments depend on the brain microenvironment, we have limited ourselves as much as possible to post-mortem and imaging studies in humans and whole-animal experiments and only describe results obtained in in vitro experiments when in vivo observations were scarce or when they were subsequently supported by in vivo data. The second part of the review deals with the general characteristics of IL-1\(\beta\) and TNF\(\alpha\) as well as their receptors before discussing in more depth their expression in the brain (peri)vascular compartments during pathology. In the final part, we discuss the role of pro-inflammatory cytokine production and action in the brain (peri)vascular compartments during bacterial meningitis, MS, HIV-1-associated dementia, ischaemic and traumatic brain injury, and Alzheimer’s disease.

### BRAIN (PERI)VASCULAR COMPARTMENTS

#### Brain fluid compartments and barriers

The brain contains different types of fluids filling different compartments. Blood vessels supply the brain with nutrients and oxygen to ascertain its high energy demand. The extracellular fluid compartment of the brain can be divided further into two compartments: ventricular CSF (cerebrospinal fluid) and parenchymal ISF (interstitial fluid). Blood constitutes the material from which CSF, that fills the brain ventricles and subarachnoid space, is made by the choroid plexi [6]. Parenchymal ISF has been proposed to be actively secreted by the capillary endothelial cells [7]. In spite of the fact that blood is the starting material for the production of CSF and ISF, blood is separated from the ISF and CSF in the CNS by the blood–brain and blood–CSF barrier respectively. These barriers are made up by cerebrovascular endothelial and choroid plexus epithelial cells that are sealed together by tight junctions and restrict the exchange between blood and CNS tissue, resulting in a more constant ion composition of the brain ISF than in the periphery. Given the specific role of ions in neural signalling, it has been argued that the advantage of more tightly controlled CNS ion homeostasis has been the foremost selecting factor leading to the evolution of the blood–brain and blood–CSF barriers [8].

The exceptions to the rule that brain endothelial cells are sealed together by tight junctions are found at the level of the circumventricular organs and the choroid plexus, where exchange between blood and ISF occurs. CSF is made from blood in the choroid plexus, meaning that blood components need to leave the blood stream in these structures. Neurons in circumventricular organs are specialized in sensing blood composition and are, therefore, in contact with blood [9]. However, the absence of tight junctions in the capillaries of circumventricular organs and choroid plexi does not mean that blood components have free access to the brain or CSF from these structures. In fact, the barrier function in these structures is displaced from capillary endothelial cells to...
epithelial cells in the choroid plexus and the ependymal cells between the circumventricular organs and the adjacent brain tissue respectively.

**Brain perivascular spaces and cells**

The vascular and perivascular basal laminae occupy perivascular spaces and form a meshwork covering the basal side of endothelial cells, smooth muscle cells, pericytes and macrophages (see below) [10]. This meshwork of basal laminae is similar to that in the general vasculature and other organs and, although it hinders diffusion, it does not constitute a barrier to the spread of signalling molecules up to several tens of thousands of kDa throughout the (peri)vascular and parenchymal basal laminae [10]. Brain perivascular spaces have been proposed to constitute a low-resistance path involved in extrasynaptic signalling and draining of surrounding brain tissue [11]. In the human brain, for instance, the arterial network in the meninges is surrounded by a sheath of leptomeninges, which is continuous with the basal lamina surrounding the penetrating arteries supplying the cortex, thus forming a periarterial space [12]. Although this sheath is continuous around arteries at the cortical surface, it becomes fenestrated as these arteries branch deeper into the cortex and is deficient at the level of capillaries. Cortical ISF may thus enter the perivascular spaces at the level of the capillaries and small arteries and drain retrogradely to the blood vessels [12]. Interestingly, arteries in the basal ganglia are surrounded by two coats of leptomeninges, thus creating a perivascular space that is not occupied by collagen of the basal lamina and through which the ISF flow is almost unhindered [12]. These perivascular spaces in the cortex and basal ganglia have been proposed as pathways through which ISF is cleared to the subarachnoid space [12].

The organization of the different cell types found in the brain (peri)vascular compartments is illustrated in the upper part of Figure 1. Pericytes are cells that are selectively associated with the microvasculature, are enclosed within the vascular basal lamina and are closely apposed to the underlying endothelial cells [13]. Pericytes are of mesodermal origin and migrate on to newly formed capillary sprouts during development before being enclosed within the basal lamina. Pericytes have been proposed to (i) have contractile properties, because at least part of them contain the muscle-specific forms of actin and myosin, (ii) regulate endothelial growth and development, and (iii) possess phagocytic activities, at least in the CNS [13]. For example, intravenous injection of high doses of horseradish peroxidase results in peroxidase activity in lysosomes of both brain endothelial cells and pericytes [14]. The possibility that processes of pericytes enter into contact with the endothelium and/or have direct contacts with the lumen of blood vessels through fenestrations in the basal lamina [15] may account for these findings.

Bone-marrow cells continuously infiltrate the CNS at a low rate to become meningeal macrophages or perivascular macrophages along vessels in the brain parenchyma, circumventricular organs and choroid plexi [16]. Perivascular macrophages lie between the vascular basal lamina surrounding endothelial cells, pericytes and the perivascular basal lamina, whereas meningeal macrophages are located immediately beneath the basal lamina covering the brain surface [10]. Brain perivascular and meningeal macrophages can be identified by their expression of CD163 [17], a haemoglobin scavenger receptor expressed exclusively by the monocyte/macrophage system [18]. The phagocytic activity of these brain perivascular and meningeal macrophages has been suggested by the presence of both endogenously and exogenously administered IgG within these cells [19].

Astrocytic endfeet are a third party in brain (peri)vascular compartments. Astrocytes, in contrast with pericytes and perivascular macrophages, are derived from neuroepithelial stem cells in the embryonic ventricular zone and can be identified by the expression of GFAP (glial fibrillary acidic protein), a filament protein [20]. Thus a large fraction of brain capillaries, but not their entire surface, is covered with astrocytic endfeet [21]. Although astrocytes are physically separated from endothelial cells by the basal lamina, they seem to provide the signals for the formation of endothelial tight junctions both during development and after blood–brain barrier breakdown provoked by tissue injury [22]. In addition to engulfing blood vessels, astrocytes enwrap neuronal synapses and have, therefore, been proposed to shuffle nutrients and metabolites between blood and neurons [23]. Finally, astrocytes play an important role in maintaining a constant ion composition of the brain extracellular fluid through their K+ -buffering capacity [24].

**Relevance of the (peri)vascular compartments to brain pathology**

Although it is easy to imagine changes in the structure and composition of brain (peri)vascular compartments in conditions that involve damage to these compartments, it may come as somewhat of a surprise that the brain (peri)vascular compartments play an important role in many neuropathologies without overt damage in these compartments. It should, however, be emphasized that disruption of the tight junctions of the blood–brain barrier and/or the presence of blood-borne molecules found in the (peri)vascular compartments that are not normally there has been described in many CNS pathologies, including bacterial meningitis, HIV-1-associated dementia, brain trauma, Alzheimer’s disease and MS.

**Bacterial meningitis**

Bacterial meningitis is characterized by the presence of colony-forming bacteria in CSF and, in spite of
Figure 1  Schematic view of the different (peri)vascular cell types involved in pro-inflammatory cytokine production and action in brain pathology

Upper panel, organization of the different (peri)vascular cell types and pro-inflammatory cytokine receptor expression under basal conditions. Cerebrovascular endothelial cells are sealed to each other by tight junctions, thus constituting a barrier between the blood and brain, excluding plasma protein from entering the CNS. Pericytes are of mesodermal origin and migrate on to newly formed capillary sprouts during development before being enclosed within the basal lamina closely apposed to the underlying endothelial cells. Perivascular macrophages find their place between the vascular basal lamina surrounding endothelial cells, pericytes and the perivascular basal lamina after the continuous low-rate extravasation of bone-marrow-derived cells. The vascular and perivascular basal laminae occupy perivascular spaces and form a meshwork similar to that in the general vasculature and other organs covering the basal side of endothelial cells, pericytes and perivascular macrophages. Astrocytes are derived from neuroepithelial stem cells in the embryonic ventricular zone and their endfeet cover a large fraction of brain capillaries. Of these different cell types, brain endothelial cells have been repeatedly shown to bear pro-inflammatory cytokine receptors under basal conditions. In addition, perivascular macrophages and astrocytes have been reported to express pro-inflammatory cytokine receptors in the absence of infection or injury, but these findings await corroboration. Lower panel, pro-inflammatory cytokine production by brain (peri)vascular cells in response to infection or insult and their putative cellular targets. Of the different cell types found in the brain (peri)vascular compartments, macrophages produce IL-1 and TNFα during bacterial meningitis, MS, HIV-1-associated dementia, Alzheimer’s disease and after traumatic and ischaemic brain injury in most of the animal models of these human brain pathologies. Pro-inflammatory cytokines produced by perivascular macrophages can potentially bind to receptors expressed by (1) endothelial cells, (2) perivascular macrophages and (3) astrocytic endfeet. In addition to perivascular macrophages, brain endothelial cells also express pro-inflammatory cytokines during bacterial meningitis, HIV-1-associated dementia and after traumatic or ischaemic brain injury. Cytokines released by endothelial cells may act (4) in an autocrine manner or in a paracrine manner on (5) perivascular macrophages, (6) monocytes and (7) astrocytic endfeet. Finally, astrocytes may not only act as targets, but also be sources of pro-inflammatory cytokines, as these cells have been reported to produce pro-inflammatory cytokines after experimental transient cerebral ischaemia and in the brains of patients with Alzheimer’s disease or its animal models.

Advances in antimicrobial therapy, is still associated with a high mortality rate, especially in newborns and the elderly. In addition, recovered patients often suffer from neurological damage, as a consequence of cerebral infarction, and sequelae including cognitive impairment [25]. In human and experimental meningitis, loss of function is due to both necrotic and apoptotic neuronal death, but the underlying pathophysiological...
mechanisms remain elusive [26,27]. Bacterial meningitis is associated with dilation of superficial cerebral blood vessels, increased blood flow, oedema and headache [28,29], which depend at least partly on substance P and prostaglandin action [30,31].

Few bacterial pathogens are capable of invading the brain by dissemination via a neural route and, in most cases, invasion of the CSF and meninges is a complication of bloodstream invasion occurring secondary to nasopharyngeal or gastrointestinal colonization [32]. In humans and animals, a high degree of bacteraemia has also been shown to be associated with meningeal invasion (for a review, see [27]). The exact routes by which high-grade extracellular pathogens cross the blood–brain and/or blood–CSF barriers and enter the CSF to give rise to meningitis have not been completely unravelled, but important progress has been made [27,33].

The tight junctions between brain endothelial cells that form the blood–brain barrier exclude paracellular diffusion of bacteria between endothelial cells. Several bacterial components constitute pathogen-associated molecular patterns that are recognized by TLRs (Toll-like receptors). TLR2 and TLR4, which recognize components of Gram-positive and Gram-negative bacteria respectively, are expressed by endothelial cells and phagocytic cells at the blood–brain interface in the choroid plexus, circumventricular organs and meninges as well as at the blood–brain barrier [34,35]. TLR2 is important for clearance of Streptococcus pneumoniae from the brain, as TLR2-deficient mice have higher bacterial loads in brain ventricles and meninges and enhanced inflammation [36].

In vitro studies with human brain microvascular endothelial cells as a model system for the blood–brain barrier have shown that Escherichia coli K1, Neisseria meningitides and group B Streptococcus invade these cells [27], suggesting that transcytosis at the blood–brain barrier is an important mechanism in infection of brain tissue and CSF in meningitis. The relative lack of parenchymal lesions, together with the frequent finding of plexitis in early meningitis, has been used as an element indicative of bacterial entry at the choroid plexus [33]. Fimbriated Haemophilus has been demonstrated to bind to human choroid plexus tissue [37]. Similarly, N. meningitides has been found interacting with the endothelium of the choroid plexus in a patient who died of meningitis [32]. These observations suggest that some extracellular bacteria frequently detected during meningitis may, in addition to invading the brain endothelial cells, enter the CSF directly at the level of the choroid plexus.

Regardless of whether bacteria cross the blood–brain or blood–CSF barriers via transcytosis, or breakdown of tight junctions, they will enter the perivascular spaces where they have to face the perivascular and meningeal macrophages as a second line of defence. It is likely that bacteria that multiply intracellularly within monocytes and macrophages, such as Mycobacterium tuberculosis and Listeria monocytogenes, invade the brain when monocytes infiltrate this organ to become meningeal macrophages and perivascular macrophages in the brain parenchyma, circumventricular organs and choroid plexus (see above) [38]. The importance of the perivascular and meningeal macrophages in host defence during bacterial meningitis is illustrated by the finding that depletion of these cells is associated with increased illness and higher bacterial counts in CSF [39]. Bacterial meningitis is also characterized by the immigration of leucocytes from the blood stream into the subarachnoid space [27]. Indeed, intravenous administration of an antiserum neutralizing adhesion molecules or chemoattractants reduces leucocyte recruitment, blood–brain barrier breakdown, cerebral oedema and mortality after intracisternal inoculation with meningitis-inducing bacteria [40,41]. Collectively, the data available in the literature indicate that host cells of the (peri)vascular brain compartments, in particular perivascular macrophages and infiltrated leucocytes, play an important role in meningitis caused by extra- and intra-cellular bacteria.

**MS**

MS was first described by Charcot in the 19th century as a perivascular accumulation of inflammatory cells in the brain and spinal cord white matter of patients with intermittent episode of neurological dysfunction [41a]. Throughout the 20th century, it has become clear that MS is a chronic inflammatory demyelinating disease that follows either a relapsing/remitting course starting between 15 and 50 years of age or initiates immediately as a progressive disorder. Classical symptoms of inflammation, pain (headache) and tissue oedema have repeatedly been reported to occur in MS [42,43]. Charcot also noticed axonal loss as an important feature of MS [41a], an observation that was recently corroborated by the early death of oligodendrocytes [44] and the accumulation of APP [Aβ (amyloid-β protein) precursor protein] in damaged axons in patients [45]. One of the functional consequences of demyelination and axonal damage is spasticity affecting gross motor activity or more refined motor skills [46]. The histopathological hallmark of MS is an area of white matter demyelination called the plaque. Active plaques occur early during disease and are characterized by the presence of mononuclear cells, including T- and B-lymphocytes and macrophages, in brain perivascular spaces. Despite the fact that no single causal agent or event has yet been identified [47], a long-favoured hypothesis in MS is that autoreactive T-cells generated in the periphery access the CNS. The animal model corroborating this hypothesis is EAE (experimental autoimmune encephalomyelitis) in which the pathological features of MS can be observed in animals after injection with myelin-containing preparations or after transfer of CD4+ T-cells [47].
The number and intensity of staining of CD163-positive perivascular macrophages and infiltrating monocytes is increased in brains of patients with MS and of animals with EAE [48,49]. Resident CNS perivascular macrophages display increased expression of adhesion molecules and chemokines for mononuclear phagocytes during EAE [50]. Interestingly, the depletion of resident brain perivascular macrophages, immunoneutralization of adhesion molecules or chemokines and knockout or pharmacological blockade of chemokine receptors alleviate the clinical signs of EAE and/or the number of lesions in MS patients [51,52]. Moreover, chemokine receptors can be found in blood-borne macrophages in the early demyelinating stages of animal EAE and human MS [53,54]. These observations indicate that resident perivascular macrophages play an important role in attracting circulating mononuclear cells. In accordance with this idea it has been shown that depletion of blood-borne macrophages reduces the number of CD163-positive brain perivascular macrophages and attenuates demyelination and the clinical symptoms of EAE [48,55]. Thus both infiltrating monocytes and resident perivascular macrophages are involved in the development of the clinical signs in this animal model of MS.

Reactive astrogliosis is another pathological feature of MS and EAE, and is characterized by hypertrophy of astrocytes and increased expression of GFAP [56]. In addition to being reactive, astrocytes can also form a scar sealing off the surrendered tissue from its environment. Scar-forming astrocytes occur mostly in the centre of chronic plaques, where repair has failed and axons are bare, whereas at the rim the tissue architecture is less disturbed and the glial scar fails to form [57]. GFAP expression is first increased around perivascular inflammatory cuffs and later during resolution of inflammation and clinical improvement [56]. The increased expression of GFAP has a protective role, as mice lacking GFAP have a more severe clinical course of EAE [58]. NGF (nerve growth factor) may play a role in the protective effects of astrogliosis, as increased levels of NGF are found in astrocytes of animals with EAE [59]. Moreover, CNS administration or expression of NGF attenuates clinical symptoms of EAE, prevents demyelination to a large extent and diminishes the perivascular infiltrates comprising extravasating monocytes and brain macrophages [60]. Taken together, these findings indicate that NGF produced by reactive astrocytes plays an important role in the remission phase of EAE and suggest that similar mechanisms are at work in MS patients.

**HIV-1-associated dementia**

HIV-1-associated dementia is the leading cause of dementia in persons between the age of 20–59 years and is characterized by progressive motor abnormalities (tremor and loss of fine motor movements), cognitive impairment (forgetfulness and poor concentration) and behavioural disorders (mania, apathy and emotional lability) [61]. It is important to point out that morphological changes in the brain, such as oedema, in AIDS patients can occur in the absence of HIV infection of the CNS [62] or as a consequence of other viral infections [63]. However, HIV-1-associated dementia is pathologically associated with HIV-1 encephalitis, which is characterized by productive viral replication in perivascular macrophages and parenchymal microglia as well as in multinucleated giant cells formed by fusion of mononuclear cells [64]. Viral infection in these cells is associated with astrogliosis, changes in white matter and dendritic arbour damage [65]. Loss of function in HIV-1-associated dementia may be due to neuronal apoptosis, which has repeatedly been shown to occur in HIV-1 encephalitis [66,67]. Apoptosis is more abundant around multinucleated giant cells [68], but also occurs at a distance from infected cells [69]. Although HAART (highly active antiretroviral therapy) has resulted in a decrease of the incidence of HIV-1-associated dementia [70], it does not provide full protection from or reversal of HIV-1-associated dementia [71]. Moreover, the prevalence of HIV-1-associated dementia may increase in the future as patients with AIDS live longer [72].

The brain (peri)vascular compartment is the main and first target in CNS infection by HIV-1, as both the viral capsid and envelope proteins can be found in brain microvascular endothelial cells and perivascular macrophages [73]. As described above, monocytes continuously infiltrate the CNS to become perivascular macrophages. The same Trojan Horse approach used by intracellular bacteria to invade the brain (peri)vascular compartments also appears to be used by HIV-1, as HIV-1 can infect monocytes and macrophages via CD4 and the chemokine receptors CXCR4 and CCR5 that function as HIV-1 coreceptors [74]. Although chemokine receptors are present on astrocytes and neurons, these cells are very poor virus producers. In accordance with this hypothesis it has been shown in monkeys that perivascular macrophages are the major cell population in the CNS infected with SIV (simian immunodeficiency virus) during peak viraemia [75]. Perivascular macrophages have also been shown to be the major virus-containing cell population in the CNS during the development of HIV-1 or SIV encephalitis [73,75]. Moreover, perivascular macrophages do not undergo apoptosis and may thus constitute a long-lasting HIV-1 reservoir in the CNS [76]. Interestingly, in humans, the presence of activated macrophages in the brain is a better predictor of neurological decline than detection of viral material [77]. Finally, the importance of brain macrophages is illustrated by the finding that local brain injection of HIV-1-infected macrophages in SCID (severe combined immunodeficient) mice induces cognitive deficits and histopathological features of HIV-1 encephalitis, such as decreased synaptic density and...
limited neuronal apoptosis at sites distant from the injection site [78].

The observation in both human and animal studies that neuronal damage can occur at some distance from HIV-1-infected cells indicate that soluble factors play a role in HIV-1-associated neuropathology. The viral protein gp120 can be shed from HIV-1 and has been found in CSF of patients with HIV-1 encephalitis and dementia [79]. Intracerebroventricular injection of gp120 induces selective neuronal apoptosis [80], and astrocytic expression of gp120 in transgenic mice also causes neuronal and glial changes reminiscent of HIV-1-associated dementia [81]. Post-mortem studies confirm the existence of a relationship between the expression of APP and HIV-1-positive brain cells [82]. APP in brain cells, but not in other organs, is expressed on the surface and is thought to act as a growth factor for neural stem cells, stimulate neurite outgrowth and modulate synaptic plasticity [83].

HIV-1 transactivator protein is a viral protein found in perivascular macrophages and endothelial cells in HIV-1 encephalitis [84] and is actively secreted by infected cells [85]. It binds to the Aβ-clearing LRP (low-density lipoprotein receptor-related protein) found on neurons and endothelial cells [86] and causes neuronal damage when injected or expressed in the brain [87]. In addition, HIV-1 transactivator inhibits the Aβ-degrading enzyme nephrilysin and thus contributes further to the accumulation of Aβ [88]. Aβ is a protein cleavage product of the APP that increases survival of hippocampal and cortical neurons in vitro at low concentrations, but is neurotoxic both in vitro and in vivo at higher concentrations [83]. As in Alzheimer’s disease (see below), brain perivascular macrophages express APP, and Aβ deposits are found around blood vessels and in diffuse plaques of patients with HIV-1-associated dementia [89]. Taken together, these observations indicate that HIV-1 transactivator protein secreted by brain perivascular macrophages plays a critical role in HIV-1-associated dementia and may favour pathophysiological processes reminiscent of those observed in Alzheimer’s disease.

Ischaemic and traumatic brain injury

Stroke is the third most common cause of death in industrialized countries and is a major cause of serious long-term disability [90]. Three major categories of stroke have been recognized: (i) subarachnoid haemorrhage, (ii) intracerebral haemorrhage and (iii) ischaemic stroke [91]. Focal ischaemia occurs with occlusion of a cerebral artery, resulting in a complete cessation of blood flow in downstream regions that do not have any collateral supply and incomplete ischaemia in the surrounding ‘penumbral’ region that is supplied by collaterals. The classical symptoms of inflammation, oedema and pain (headache) have repeatedly been reported to be associated with stroke [92,93]. It is important to point out, though, that oedema, and probably pain, early after stroke are the consequence of blood–brain barrier breakdown due to the lack of ATP after interruption of blood flow and to free radical formation after reperfusion [93], rather than the consequence of inflammatory reactions.

Ischaemic brain injury is the consequence of a complex sequence of events starting with a decrease in blood flow, leading to a reduction in cellular ATP that is required to maintain ionic gradients. The disruption of ionic gradients, in turn, leads to an influx of Na⁺ and Ca²⁺, cellular depolarization and release of neurotransmitters, including the excitotoxin glutamate. As energy-dependent removal of glutamate is impaired, this excitatory neurotransmitter leads to overactivation and opening of monovalent ion channels followed by water influx, thus resulting in cellular swelling [94]. Although oxygen and glucose deprivation is responsible for immediate neuronal injury after ischaemia, cell death continues once blood flow has been restored. The formation of reactive oxygen species that occurs during reperfusion after ischaemia contributes further to neuronal death [95], but also to loss of blood–brain barrier integrity and vasogenic oedema [96].

Cerebral microvessels have long been considered as less sensitive to ischaemia than neurons, but accumulating evidence now indicates that the downstream microvascular bed shows important changes following focal ischaemia with recovery of flow in the occluded arteries. Indeed, the first hours after middle cerebral artery occlusion are associated with a loss of blood–brain barrier integrity and the underlying extracellular matrix, expression of leucocyte adhesion molecules and swelling of astrocytic endfeet in the downstream microvasculature [97]. Gap junctions between astrocytes may play an important role in diluting excess ions and neurotransmitters that were taken up by astrocytes at the site of the infarct. In accordance with this idea, mice deficient in one of the gap-junction molecules have been shown to be more resistant to ischaemic stroke [98]. However, severe astrocytic swelling causes oedema, compromising blood flow further and mediating astrocytic cell death. In this manner, astrocytic gap junctions may contribute to the spreading of apoptotic or necrotic signals thus exacerbating cellular damage after cerebral ischaemia [99].

Although astrocytes are mostly necrotic in the infarct area itself, they are viable in the penumbral region and show reactive astrogliaosis around the penumbra. Reactive astrogliosis is due to hypertrophy and to a lesser extent proliferation [100] and is accompanied by an accumulation of intermediate glial filaments consisting mostly of GFAP [101], enlarged nuclei and an increased number of mitochondria and ribosomes. Interestingly, mice genetically deficient in GFAP display greater infarct volume, decreased local blood flow and increased intracranial blood flow after ischaemia compared with controls [102], suggesting that astrogliosis is important for neuronal survival.
Blood-borne neutrophils are attracted early on to the infarct area after ischaemia in humans [103]. Given that neutrophils in other tissues can cause damage, these cells have received a lot of attention in ischaemia research. Moreover, these cells are present in the infarct area at the time that neuronal death occurs, and treatments targeted to interfere with leucocyte adhesion and extravasation have sometimes shown beneficial effects. However, it has also been argued that, since few neutrophils are present before maximal damage [104], they may at best play a role in neuronal damage during the end stage of infarction [105]. Although overexpression of MCP1 (monocytic chemoattractant protein 1) in the brain results in increased perivascular accumulation of neutrophils after ischaemia and is associated with larger infarct volume [106], it has been much harder to convincingly show that treatments reducing infarct size do so because of their effects on neutrophils. Thus it seems too early to draw any firm conclusions concerning the role of infiltrated neutrophils in mediating neuronal damage after ischaemia.

An increase in GFP (green fluorescent protein)-positive macrophages in the brain can be observed 3–4 days after ischaemia in mice transplanted with GFP-expressing leucocytes and is most intense at 7–10 days, most probably due to the recruitment of blood monoocytes [107]. Brain macrophages, in addition to dystrophic axons, also express APP and Aβ several days after experimental ischaemia is induced by temporal occlusion of the middle cerebral artery in rats [108]. Interestingly, microvascular accumulation of APP and Aβ penetrating into the surrounding parenchyma can still be observed 1 year after brain ischaemia due to cardiac arrest [109]. These observations are reminiscent of those made in some cases with Alzheimer’s disease and suggest that cerebral ischaemia plays a role in Alzheimer’s disease (see below, and [110] for a more detailed discussion).

Bone-marrow-derived cells also play a role in the formation of new blood vessels after cerebral ischaemia and, thus, contribute to the restoration of blood flow to damaged brain areas [111]. Although the majority of the infiltrated cells express macrophage markers and do not proliferate, a small subset of bone-marrow-derived cells do proliferate and give rise to pericytes expressing VEGF (vascular endothelial growth factor) [112]. This suggests that brain pericytes are recruited from the periphery and are involved in vessel stabilization during ischaemia-induced angiogenesis.

In industrialized countries, traumatic brain injury is common, with an estimated incidence of 1:1000, and represents the leading cause of death and neurological impairment in patients under the age of 45 years [113]. The most common type of traumatic brain injury is contusion, which is characterized by a short-lasting disturbance of neural function provoked by a sudden acceleration or deceleration of the head and is associated with a short loss of consciousness as well as amnesia [114].
compartments, traumatic injury provokes abnormalities and lesions of the endothelium [132], pericyte migration into the neuropil accompanied by degeneration of non-migrating pericytes [133], neutrophil infiltration [131], increases in the number of perivascular macrophages [134] and the loss and swelling, followed by proliferation, of astrocytes [119,135,136].

The relevance of cellular changes in the (peri)vascular brain compartments to neuronal damage are at present unclear. Although the infiltration of neutrophils has been shown to damage the blood–brain barrier, this does not seem to be the case after traumatic brain injury caused by fluid percussion injury, since neutrophil infiltration occurs after maximal blood–brain barrier permeability [137]. Although neuronal degeneration is most important in the hippocampus and cortex where the blood–brain barrier is permeable and neutrophils infiltrate [131], it also occurs in regions with an intact blood–brain barrier [131]. Finally, inhibiting neutrophil infiltration by the administration of antibodies neutralizing leucocyte adhesion molecules does not improve neurological outcome [138]. Taken together, these observations indicate that neutrophil infiltration is not necessarily responsible for blood–brain barrier breakdown and neuronal death after traumatic brain injury.

The migration of pericytes into the parenchyma has been speculated to play a role in the guidance of new vessel formation, since similar pericytic responses have been observed in response to angiogenic stimuli [139]. As mentioned above, proliferation of astrocytes occurs after the initial loss of astrocytes in response to traumatic injury [136]. Interestingly, preventing astrocytic proliferation results in widespread tissue disruption, more pronounced cellular degeneration and severe persisting motor deficits after a short-lasting crush injury of the spinal cord [140]. Pericytes and astrocytes thus seem to play an important role in the recovery of function after traumatic injury to the CNS.

Alzheimer’s disease

Alzheimer’s disease is the most frequent cause of dementia and manifests itself as a progressive disease affecting higher brain functions, including memory, problem solving, planning and abstract thought [141]. Two forms of Alzheimer’s disease exist. Sporadic Alzheimer’s disease can affect adults of either sex, whereas familial Alzheimer’s disease is caused by rare genetic mutations. So far, none of the classical symptoms of inflammation, such as headache, brain oedema or vasodilatation, have been described in patients with Alzheimer’s disease.

Although the main focus in research on Alzheimer’s disease over the last decades has been on the neurotoxicity of fibrillar Aβ accumulation, it is important to point out that this pathology is also characterized by elevated levels of Aβ in the (peri)vascular compartments [142,143]. Aβ is a protein cleavage product of APP, a molecule that is widely expressed in a variety of cell types, including neurons. APP is expressed on the surface of brain cells, but not in other organs, and is thought to act as growth factor for neural stem cells to stimulate neurite outgrowth and to modulate synaptic plasticity [83]. Mice engineered to express high levels of Aβ(1–40) do not develop amyloid pathology, whereas lower expression of Aβ(1–42) does result in insoluble amyloid deposits in the parenchyma and around vessels [144], indicating that the latter fragment is probably essential in the development of Alzheimer’s disease. Aβ is cleared from the brain by (i) transport systems involving LRP1 at the abluminal side of the blood–brain barrier [145], (ii) uptake by astrocytes, (iii) perivascular drainage [12], and (iv) proteolytic degradation by neprilysin in brain parenchyma [146]. Interestingly, plasma Aβ is transported across the blood–brain barrier by RAGE (receptor for advanced glycation products), and has been shown to reach the (peri)vascular compartments [147], suggesting that the blood Aβ pool can be a contributing factor to brain Aβ levels, especially if clearance is compromised. One of the mechanisms through which circulating Aβ may contribute to Alzheimer’s disease is cerebral vasoconstriction and increased vascular resistance, resulting in cerebral hypoperfusion [148].

Cerebral amyloid angiopathy is characterized by deposits of amyloid protein in the vessel walls of large meningeal and parenchymal blood vessels and/or (pre)capillaries and is frequently seen in Alzheimer’s disease. Indeed, among cases with cerebral amyloid angiopathy the density of blood vessels with amyloid deposits is 2.5–4 times higher in patients with Alzheimer’s disease than in age-matched controls [149]. Interestingly, the APOE (apolipoprotein E) genotype, another ligand of LRP, influences cerebral amyloid angiopathy. In particular, the APOEε4 allele is associated with increased prevalence and severity of cerebral amyloid angiopathy and reduced longevity in Alzheimer’s disease [150,151]. Finally, cerebral amyloid angiopathy appears to correlate more strongly with dementia than other pathological features of Alzheimer’s disease [142].

Expression of APP can be found in smooth muscle cells of meningeal vessels and parenchymal arterioles as well as in endothelial cells, pericytes and perivascular macrophages of capillaries in both familial and sporadic forms of Alzheimer’s disease [152,153]. Smooth muscle cells are a source of Aβ in large meningeal and parenchymal vessels and can cause degeneration of muscle cells, reduced thickness of the smooth muscle layer and rupture of the vessel wall leading to bleeding [153].

It has recently been shown in a mouse model expressing a mutant form of APP found in familial form of Alzheimer’s disease that perivascular macrophages contain cytoplasmatic channels filled with fibrillar amyloid [154]. Perivascular macrophages also produce the fibrillar form of amyloid around capillaries in sporadic...
Alzheimer’s disease [153]. Amyloid was found to infiltrate adjacent basal lamina and to accumulate on the outer side of the basement membrane of capillaries in Alzheimer’s disease and its animal models [154,155]. Deposits of amyloid in (pre)capillaries by perivascular macrophages cause damage to the basal laminae, degeneration of the endothelium and obliteration of the lumen, leading to local ischaemia and neurodegeneration [153]. Fibrillar amyloid deposits on the vessel wall can grow into the neuropil where they are surrounded most of the time by astrocytic processes. In areas where amyloid breaks through these gial processes, neurite dystrophy occurs, indicating that angiopathy has damaging effects on neurons [153,154]. Collectively, these findings indicate that A\(\beta\) production by brain perivascular macrophages and the associated cerebral amyloid angiopathy play an important role in the pathogenesis of both familial and sporadic Alzheimer’s disease [142,143].

EXPRESSION OF PRO-INFLAMMATORY CYTOKINES AND THEIR RECEPTORS IN BRAIN (PERI)VASCULAR COMPARTMENTS

Is the brain immune to immunity? The brain was long considered an immunologically privileged organ, based on the presence of the blood–brain barrier and the lack of professional antigen-presenting cells, such as dendritic cells, as well as of a lymphoid system in the brain parenchyma. This is evolving now as dendritic cells have been found in circumventricular organs, meninges and choroid plexus as well as associated with vessels making up the blood–brain barrier [156,157]. Interestingly, increased numbers of dendritic cells have been observed in brains of patients with MS and in the CNS of animals suffering from EAE or ischaemia [157,158]. Moreover, it has recently been shown that augmenting the number of dendritic cells increases the clinical severity of EAE induced by the transfer of encephalitogenic T-helper cells [157]. Furthermore, in spite of the absence of a true lymphoid system in the brain parenchyma, there is evidence indicating that antigens presented in the ISF drain via peri-arterial spaces and CSF to lymph nodes [12,159]. In addition, it has recently been shown that antigen-loaded dendritic cells injected into the brain can migrate to cervical lymph nodes and initiate specific T-cell homing to the CNS [160]. These findings indicate that dendritic cell migration throughout perivascular brain compartments plays an important role in immune invasion of the CNS in T-cell-dependent neuropathologies. However, given the lack of experimental data available to date, we will not address further the role of dendritic cells in the present review.

When antigens are introduced into the brain parenchyma, they do elicit transient innate immune responses, including the release of cytokines, and the recruitment of monocytes and neutrophils in spite of the blood–brain barrier [161]. Research in this fast-moving field has now convincingly shown that, albeit different from the periphery, circumventricular organs, ventricles and meninges, inflammatory responses are mounted in the brain parenchyma [162].

General features of pro-inflammatory cytokine production and action

Before addressing the specific role of IL-1 and TNF\(\alpha\) in the (peri)vascular brain compartments during CNS pathology, it is important to point out some of the general characteristics of IL-1 and TNF\(\alpha\) synthesis and action. IL-1 and TNF\(\alpha\) are cytokines, which can be defined as regulatory proteins controlling survival, growth and effector functions of tissue cells. Cytokines are produced by many different cell types. They require de novo synthesis before release and are often not stored in vesicles. Once released, cytokines mostly act locally in an autocrine or paracrine manner at low concentrations. As various cell types express their receptors, cytokines have a wide variety of effects (pleiotropy). Finally, different cytokines can provoke the same biological effects (redundancy). These general features of cytokines have consequences for the study of IL-1 and TNF\(\alpha\) production and action in the (peri)vascular brain compartments.

IL-1

The IL-1 family has three well-known endogenous ligands, two agonists, IL-1\(\alpha\) and IL-1\(\beta\), and one IL-1R (IL-1 receptor) antagonist, IL-1ra. A number of recently discovered molecules related to classical IL-1 family members have been found to be expressed in the brain or brain-derived cells; however, as relatively few studies have addressed the role of these new IL-1 family members in brain pathology, the present review will focus mostly on the role of IL-1\(\alpha\) and -\(\beta\).

Although IL-1\(\alpha\) and IL-1\(\beta\) both signal through IL-1R1 (type 1 IL-1R) [163] and in general induce similar effects when administered exogenously, their physiological roles probably differ. IL-1\(\alpha\) and IL-1\(\beta\) are produced as immature pro-peptides upon transcription of different genes, but differ in their maturation and secretion. Both pro-peptides lack hydrophobic leader sequences and remain in the cytosol [164]. Pro-IL-1\(\alpha\) can be cleaved by calcium-dependent membrane proteases to yield mature IL-1\(\alpha\) [165]; however, IL-1\(\alpha\) rarely appears in extracellular biological fluids and, when it does, it is thought to originate from lysed cells and cleavage by extracellular proteases [166]. As pro-IL-1\(\alpha\) is just as active as mature IL-1\(\alpha\) and appears to remain intracellular or on the surface of mononuclear cells, IL-1\(\alpha\) is thought to act intracellularly or via cell-to-cell contact [167,168].

Although most pro-IL-1\(\beta\) is present in the cytosol, a fraction moves into specialized secretory lysosomes,
where it co-localizes with pro-caspase 1 [169]. During the initiation of IL-1β synthesis, pro-caspase 1 is cleaved to caspase 1 or ICE (IL-1β-converting enzyme) which, in turn, cleaves pro-IL-1β to yield mature IL-1β [170]. The exact route by which IL-1β is secreted is still a matter of debate, but involves activation of the purinergic P2×7 receptor [171]. The action of IL-1 on IL-1R1 is negatively regulated by IL-1ra and soluble IL-1Rs that are processed by extracellular matrix proteins induced by IL-1 [172] and other pro-inflammatory cytokines. To provoke biological responses, IL-1R1 has to associate with the IL-1R AcP (accessory protein) to recruit the adapter protein MyD88 [173]. MyD88 via other molecular intermediates can interact with TRAFs [TNFR (TNF receptor)-associated factors] which, in turn, gives rise to the phosphorylation of IkB (inhibitory-κB), MAPKs (mitogen-activated protein kinases) and JNK (c-Jun N-terminal kinase), resulting in the activation of NF-κB (nuclear factor κB), AP1 and C/EBPβ (CCAAT/enhancer-binding protein β) transcription factors [174].

TNF
In general, TNF can be considered a major pro-inflammatory cytokine with an optional capacity to induce apoptosis [175]. TNF can be active both as a membrane-integrated [175] and a soluble molecule after its processing by the metalloprotease TACE (TNFα-converting enzyme) [176]. Membrane-bound and/or soluble TNF interact with two specific receptors with a molecular mass of 55–60 and 75–80 kDa respectively, that are either membrane-anchored or soluble. The p75 TNFR, in contrast with the p55 receptor, can only be fully stimulated by the membrane-integrated form of its ligand, rather than by soluble TNF [177]. Upon stimulation, TNFRs, in particular the p75 type, can be cleaved from the membrane or are directly expressed as soluble isoforms lacking the transmembrane domain [178]. As with TNF itself, p75 TNFRs are released by TACE [179]. The p75 TNFR contains TRAF-interacting motifs that recruit TRAFs and thus directly activate the same intracellular pathways as IL-1 [180]. TRADD (TNFR-associated death domain) is an adaptor protein associated with the p55 TNFR that induces apoptosis when protein synthesis in a cell is blocked [181]. TRADD competes with a molecule coined MADD (MAPK-activating death domain) for binding to the intracellular portion of the p55 TNFR and activation of MAPK and NF-κB [182,183]. Several MADD splice variants exist that render permanently transfected cells susceptible or resistant to TNFα-induced apoptosis [183]. When protein synthesis is not blocked, TRADD interacts with TRAFs to give rise to the phosphorylation of IkB, MAPK and JNK, resulting in the activation of NF-κB and AP1 transcription factors, which protect cells from apoptosis and induce the transcription of inflammatory genes [184,185].

Pro-inflammatory cytokine production in brain (peri)vascular compartments
Clinical studies on CSF composition have provided the first hint to the production of pro-inflammatory cytokines in the CNS in response to infection or injury. Indeed, increased CSF concentrations of IL-1 and/or TNFα have been reported in patients suffering from HIV-1-associated dementia, Alzheimer’s disease, bacterial sepsis, meningitis, head injury, MS and stroke [186–192]. Since the initial demonstration of IL-1α immunoreactivity in brain vascular and glial cells in Alzheimer’s disease, several studies conducted both on human patient material and in animal models have shown the synthesis of IL-1 and TNF in the (peri)vascular compartments of the CNS, as illustrated in Figure 1. In patients who died from meningitis, IL-1β immunoreactivity was observed in cells positive for CD68 [193], a marker expressed by perivascular macrophages and infiltrating monocytes [49], whereas TNFα was expressed by endothelial cells [193]. TNF-α mRNA, and to a lesser extent IL-1α mRNA, has been found in inflammatory cuffs of brains obtained from patients with MS, with both cytokines being expressed strongly in (peri)vascular cells [194]. IL-1-immunoreactive macrophages have been found in the centre of MS lesions, whereas TNF-α immunoreactivity can be observed in perivascular macrophages, astrocytes and endothelial cells [195,196]. Brain endothelial cells in autopsy material from the cerebral cortex and white matter of HIV-seropositive patients are frequently immunoreactive for IL-1 and less frequently so for TNF-α [197]. IL-1α can also be detected in perivascular macrophages, whereas TNF-α mRNA and immunoreactivity are mostly found in these cells in brain tissue of patients with HIV-1-associated dementia [198,199]. In situ hybridization and immunohistochemistry for IL-1β in autopsy material from patients operated for loss of function due to brain trauma revealed preferential expression of IL-1β in endothelial cells, mononuclear phagocytes and microglia [200]. Finally, brain microvesels isolated from patients with Alzheimer’s disease contain higher levels of IL-1β and spontaneously secrete more of this cytokine as well as of TNFα than vessels obtained from controls [201]. In parallel, in an animal model of bacterial sepsis, it was shown that intravenous administration of high doses of bacterial LPS (lipopolysaccharide) in the rat induces IL-1β mRNA and immunoreactivity in (peri)vascular cells at the blood–brain interface [202,203]. However, peripheral injection of a high dose of bacterial LPS results in TNF mRNA induction in parenchymal microglia, but not in perivascular macrophages [204]. Moreover, experimental meningitis induced by intracerebral injection of group B Streptococcus provokes IL-1β, but not
TNFα, mRNA induction associated with cortical blood vessels [205]. However, TNFα mRNA induction in the meninges after experimental meningitis induced by intracisternal S. pneumoniae occurs in cellular infiltrates and is prevented by peripheral depletion of phagocytic cells [206], suggesting that infiltrated perivascular macrophages produce TNF in the meninges. In EAE, the animal model of MS, IL-1β and TNF immunoreactivity can be found in mononuclear cells in perivascular lesions in the rodent brain [207,208]. In an EAE model in monkeys, perivascular infiltrates stain positively for IFNγ (interferon γ), IL-1α, IL-1β and TNFα [209].

More recent studies employing intracerebroventricular injection of fluorescent markers have shown that IL-1β is expressed by resident brain perivascular macrophages during EAE in rodents [50]. TNF production during EAE, on the other hand, appears to be due mainly to infiltrated monocytes, as peripheral depletion of phagocytic cells inhibits TNFα mRNA in the brain of mice injected with myelin-reactive T-cells [55]. Demyelination also appears to be a sufficient signal for IL-1β induction, as cuprizone-induced demyelination results in IL-1β immunoreactivity in brain macrophages and astrocytes several weeks later [210]. Intracerebroventricular injection of the HIV protein gp120 induces IL-1β immunoreactivity in cellular elements around parenchymal blood vessels [211]. Furthermore, local injection of the HIV-1 transactivator molecule into the hippocampus results in TNF-α immunoreactivity in perivascular macrophages [212]. Experimental traumatic brain injury results in both IL-1β and TNFα immunoreactivity in mononuclear phagocytic cells at the site of the lesion, and also from the lesion around blood vessels [213]. In a similar vein, after transient forebrain ischaemia in rodents, IL-1β and/or TNFα mRNA and immunoreactivity are induced in endothelial cells, perivascular macrophages and astrocytes of the cerebral cortex, hippocampus, striatum and thalamus [214–216]. In transgenic mice expressing a mutant form of APP found in familial Alzheimer’s disease, astrocytes surrounding early amyloid plaques are IL-1β-immunoreactive [217].

From this wide variety of anatomical observations, it is evident that the expression of pro-inflammatory cytokines in (peri)vascular cells is a common feature of different types of experimental and clinical neuropathologies.

**Pro-inflammatory cytokine receptor expression in brain (peri)vascular compartments**

The expression of pro-inflammatory cytokines in (peri)vascular cells in different types of neuropathologies gives rise to the question as to where these cytokines act in the brain. Studies employing radioactive *in situ* hybridization have shown constitutive expression of mRNA coding for IL-1R1 and p55 TNFR to be associated with blood vessels in the rodent brain [218–220]. Subsequent studies using PCR have shown that IL-1R1 and p55 and p75 TNFR mRNA are present in cultured rodent cerebrovascular endothelial cells [221–223]. Moreover, cultured rodent cerebrovascular endothelial cells bind radioactively labelled IL-1 and TNFα, suggesting that at least endothelial cells express pro-inflammatory cytokine receptors [222,223]. A recent study confirmed that IL-1R1 mRNA expression was associated with blood vessels in the rodent brain and showed, by double-labelling immunohistochemistry with an antiserum raised against the extracellular part of the rat IL-R1, that both endothelial cells and perivascular macrophages constitutively express this receptor [224]. Immunohistochemical analysis of post-mortem normal human brain material with monoclonal antibodies revealed some weakly IL-1R1-immunoreactive endothelial and microglial cells [225]. A similar pattern was observed for p55 and p75 TNFR immunoreactivity in normal human brain. In contrast, a case study of a normal human brain stained with a polyclonal antibody revealed strong IL-1R1 immunoreactivity in cells with astrocytic morphology as well as (peri)vascular cells [226]. From these anatomical findings, it can be concluded that the IL-1R1 and p55 and p75 TNFRs are constitutively expressed by brain endothelial cells. Although the observations need to be confirmed, brain perivascular macrophages and astrocytes also appear to contain pro-inflammatory cytokine receptors.

Before discussing the regulation of pro-inflammatory cytokine receptor expression in the brain (peri)vascular compartments during neuropathology, it is important to point out that these receptors are also expressed by neurons. Indeed, the developing and adult olfactory bulb, hippocampus and some brainstem motor neurons express IL-1R1 and p55 TNFR mRNA in rodents and amphibians [219,227]. These observations suggest that some of the effects of IL-1 and TNFα produced by (peri)vascular cells in these structures during neuropathology might be brought about by a direct action of these cytokines on neurons.

Increased expression of the IL-1R1 and p55 TNFR in the brain (peri)vascular compartments has been observed in several pathologies affecting the CNS. High doses of peripherally injected bacterial LPS result in increased expression of IL-1R1 in the brain, as measured by PCR, and of p55 and p75 TNFRs at the blood-brain and blood-CSF interfaces [220,228]. The expression of both the p55 and p75 TNFRs is increased in high endothelial CNS venules before the onset of the clinical symptoms of EAE [229]. Double-labelled immunohistochemistry on brain material from patients with AIDS revealed a prominent up-regulation of p55 and p75 TNFRs on activated macrophages in patients with HIV-1 encephalitis [230]. After forebrain ischaemia due to middle cerebral artery occlusion in rodents, PCR analysis revealed a rise
in IL-1R1 mRNA expression in the brain cortex [231]. However, apart from up-regulation of IL-1R1 mRNA and immunoreactivity in hippocampal neurons, no anatomical study has been able to show an increase in IL-1R expression over basal levels in the (peri)vascular compartment after forebrain ischaemia [232]. In contrast, increases of p55 TNFR mRNA and immunoreactivity have been described both in the (peri)vascular compartment as well as in parenchymal neurons after forebrain ischaemia [233]. Within the (peri)vascular compartment p55 TNFR immunoreactivity was detected in astrocytes and perivascular macrophages [215,233]. Thus, in addition to the induction of IL-1 and TNFα in different types of neuropathologies, their receptors are also constitutively expressed in the (peri)vascular compartment and, in some cases, even up-regulated.

The lower part of Figure 1 provides a summary of the different brain (peri)vascular cells expressing pro-inflammatory cytokines and their receptors, reflecting the putative modes of cytokine action in brain pathology.

**ACTION OF PRO-INFLAMMATORY CYTOKINES IN BRAIN (PERI)VASCULAR COMPARTMENTS**

**Bacterial meningitis**

The level of TNF production in healthy individuals shows a wide variation, with high- and low-producing phenotypes. In patients with a fatal meningococcal disease, high ratios of TNFα over its soluble receptors are associated with survival [234]. Experimentally, TNFα was first related to meningeal inflammation when it was shown that intracisternal administration of homologous TNFα in rabbits increases CSF white blood cell counts [235]. Conversely, intracisternal administration of neutralizing IL-1β antibodies reduces CSF white blood cell counts [239]. Conversely, intracisternal administration of neutralizing IL-1β antibodies reduced increased CSF white blood counts in animals with *H. influenza* meningitis [239]. Peripheral administration of a caspase 1 inhibitor in rats or caspase 1 gene deletion in mice, resulting in lower CNS IL-1β concentrations, reduced expression of leucocyte-attracting chemokines, lowered CSF leucocyte counts, attenuated intracranial pressure and improved clinical score [240]. These observations indicate that IL-1 production by, and action on, brain endothelial cells and perivascular macrophages (see above) plays an important role in attracting leucocytes and mediating increased intracranial pressure during meningitis. However, (peri)vascular IL-1 action is indispensable during the early host response, as IL-1R1-deficient mice have reduced bacterial clearance from CSF and increased mortality after intranasal inoculation with *S. pneumonia* [241].

**MS**

Several studies have addressed the relationship between polymorphisms in the TNFα promoter and clinical features of MS. With the exception of two Spanish studies showing that the frequency of an allele affecting transcription factor binding to the TNF promoter [242] is increased in MS patients [243,244], none of the polymorphisms in TNFα have been found to be associated with the incidence of the relapsing form of MS [245,246]. The first experimental indication that TNFα plays a role in EAE was provided by the observation that peripheral TNFα administration prolongs EAE and induces relapses [247,248]. This effect is probably due to a central site of action, as central TNFα administration enhances axonal damage and exacerbates clinical symptoms of EAE [249]. Interestingly, astrocytic, but not neuronal, overexpression of membrane-bound TNFα results in up-regulation of adhesion molecules, infiltration of T-cells and macrophages, CNS loss of myelin mass and axons, and paralysis and is thus reminiscent of EAE [250]. Collectively, these observations indicate that increased membrane expression of TNFα by astrocytes is sufficient to induce up-regulation of adhesion molecules, infiltration of macrophages and T-cells, demyelination and EAE-like symptoms.

Conversely, blockade of TNFα action with neutralizing antibodies or soluble p55 TNFRs reduces cellular infiltration or the activation state of cellular infiltrates and resident brain macrophages and prevents or alleviates clinical signs of EAE [251–254]. In accordance with the findings obtained by these pharmacological approaches, mice genetically deficient for TNFα have delayed EAE...
onset, reduced chemokine expression by brain macrophages, impaired leucocyte infiltration and less perivascular cuffs formed by leucocytes [255]. The effects of TNFα produced by brain perivascular macrophages at the onset of EAE appear to be mediated through p55 TNFR, as mice lacking this receptor also display delayed EAE onset [256] and less demyelination [257]. However, severe EAE characterized by paralysis, CNS perivascular inflammation and demyelination does eventually develop in the absence of TNFα [255]. In addition to the evidence showing that TNFα plays an important role in EAE onset, TNFα is also known to promote proliferation of oligodendrocytes and remyelination following demyelination induced by cuprizone administration [258] and might thus play a role in recovery from EAE. This possibility is corroborated by observations that administration of some anti-TNFα preparations and knocking-out of the TNFα gene in mice result in exacerbated EAE [253,259]. Since p75 TNFR knock-out mice have less oligodendrocyte regeneration after cuprizone administration [258] and develop a chronic form of EAE with a very severe onset [260], it appears that the regenerative effects of TNFα are mediated via this receptor.

Although conflicting data exist, polymorphisms within the IL-1 family cluster of genes have been associated with MS [261,262]. It is clear, however, that families characterized by high IL-1β/IL-1ra production ratio are at a double risk of having patient relatives with relapse-onset MS than families with a low ratio [263]. As with TNFα, the first experimental evidence indicating that IL-1 plays a role in the clinical signs of EAE consisted of the observation that peripheral IL-1α administration exacerbated EAE [264]. Moreover, chronic expression of IL-1β by a recombinant adenovirus injected into the brain parenchyma induced demyelination [265]. Conversely, pre-treatment with IL-1ra or soluble IL-1Rs delayed the onset and reduced the severity and duration of EAE [264,266]. Similarly, mice with a deletion of IL-1R1 failed to develop inflammatory CNS lesions and clinical symptoms of EAE [256]. However, starting IL-1ra treatment at the day of EAE onset does not improve the clinical course of the disease [267]. On the contrary, after demyelination induced by cuprizone administration, IL-1β-deficient mice fail to remyelinate properly, probably because oligodendrocytes do not mature in these animals [210]. These findings indicate that IL-1 produced by perivascular macrophages and acting on its signalling receptor on endothelial cells and macrophages (see above) plays an important role in EAE onset and potentially in recovery through an action on oligodendrocyte precursors.

**HIV-1-associated dementia**

Although the frequency of an allele associated with higher TNF production is increased in patients with HIV-1-associated dementia compared with HIV-1 infection without dementia [268], this allele is not associated with autopsy proven HIV-1 encephalitis [269]. These observations can be interpreted to suggest that, although TNF produced by brain perivascular macrophages plays an important role in the early neurological changes underlying HIV-1-associated dementia, other factors determine full-blown encephalitis at death. Experimental data showing that peripheral administration of an inhibitor of matrix metalloproteases and TACE attenuates expression of adhesion molecules, astrogliosis and axonal damage in SCID mice during the first week after intracerebral injection of HIV-1-infected human macrophages [270] corroborates this view.

Similarly to TNF, no association could be established between polymorphic alleles of IL-1β and the pathological characteristics of HIV-1 encephalitis at autopsy [269]; however, it has been shown experimentally that intracerebroventricular infusion of the IL-1ra prevents cortical neuronal apoptosis induced by gp120 administration [211]. Although these data suggest that IL-1 produced by (peri)vascular cellular elements plays a role in HIV-1-associated neuronal damage, further research is needed to establish the role of IL-1 in the pathophysiology of HIV-1-associated encephalitis and dementia.

**Ischaemic and traumatic brain injury**

To date, no studies have established a link between a TNF or IL-1 allele and the onset or increased risk for ischaemic brain injury. One of the first experimental intervention studies addressing the role of IL-1 in brain injury showed that neuronal death is inhibited in rats injected with 10 μg of recombinant human IL-1ra into the lateral brain ventricle just before and after focal cerebral ischaemia [271]. Similarly, intracerebroventricular administration of an ICE/caspase 1 inhibitor just before transient cerebral ischaemia attenuates the increase in brain IL-1β immunoreactivity, reduces infract volume and improves neurological score [272].

Importantly, intracerebroventricular administration of recombinant human IL-1ra also reduces infarct volume in rat brain when given several hours after cerebral ischaemia [273], suggesting that IL-1 plays a role in cell death once blood flow has been restored. The involvement of IL-1 in mediating brain injury and neurological outcome after cerebral ischaemia has been supported further by studies showing that mice deficient in IL-1α/IL-1β, ICE or IL-1R1 have less important infarcts, reduced cerebral oedema, attenuated vascular expression of adhesion molecules, lower numbers of brain macrophages, increased neuronal survival and preserved sensorimotor function [274–276]. Taken together, these observations can be interpreted to suggest that, after ischaemia, IL-1 produced by endothelial cells, perivascular macrophages and astrocytes acts on IL-1Rs on endothelial cells and perivascular macrophages (see above) to provoke the expression of adhesion molecules and leucocyte influx.
which, in turn, influence neuronal survival and neurological outcome.

More than 10 years ago, it had already been shown that systemic administration of IL-1ra either before or several times after ischaemia limited infarct volume in different rat models of cerebral ischaemia [277]. Moreover, in addition to decreasing the number of necrotic neurons, peripheral administration of IL-1ra reduced the number of leucocytes in the brain and improved neurological motor score after middle cerebral artery occlusion in rats [278]. These observations paved the way for an ongoing clinical trial addressing the effect of peripheral IL-1ra administration on outcome of human stroke patients, which is seemingly successful [279].

Genetically engineered mice expressing transmembrane TNFα in astrocytes had an increased expression of p55 TNFRs on parenchymal glial cells and p75 TNFRs on the brain vasculature [280]. However, p75 TNFR-knockout mice do not differ in infarct volume compared with wild-type mice after focal cerebral ischaemia/reperfusion [281], indicating that TNFα receptor signalling through its p75 TNFR is not necessary for ischaemic brain damage. Interestingly, p55 TNFR and double p55/p75 TNFR-knockouts had even higher infarct volumes than wild-type and p75 TNFR-deficient mice [281], suggesting that this TNFR plays a protective role. However, studies with TNF-knockout mice show no differences in neuronal death in the hippocampus after global transient ischaemia [282], perhaps because TNF acts at its two receptor subtypes at different time points after reperfusion. In accordance with this possibility, studies employing classical intervention strategies have shown that early central or peripheral administration of TNFα-neutralizing antibodies, soluble TNFRs or small-molecule TACE inhibitors reduce cerebral oedema, protect against ischaemic brain injury and improve motor function recovery [283–286]. Taken together, these findings indicate that TNFα produced by endothelial cells, perivascular macrophages and astrocytes (see above) induces neuronal death as well as motor and cognitive deficits in the first days after cerebral ischaemia. At later time points TNFα action appears to reduce infarct size by acting on its p55 receptor.

The first experimental study addressing the role of IL-1 in traumatic brain injury showed that repeated intracerebroventricular administration of IL-1ra reduced cortical damage after fluid percussion trauma [287]. However, intracerebroventricular administration of similar doses of IL-1ra was associated with a tendency to exacerbate motor dysfunction after traumatic brain injury [288]. Interestingly, subcutaneous administration of IL-1ra also attenuates neuronal loss in the hippocampus and cognitive deficits after experimental traumatic brain injury, but impairs recovery of motor function at high doses [289]. These observations indicate that, after traumatic brain injury, IL-1 produced by perivascular brain macrophages [213] acts on IL-1Rs expressed by endothelial cells, perivascular macrophages and hippocampal neurons (see above) to induce neuronal death in the hippocampus and cortex leading to cognitive impairment, but also to promote recovery of motor function.

In contrast with ischaemic brain injury, intracerebroventricular administration of TNFα-neutralizing antibodies does not affect motor and cognitive impairment during the first week after fluid percussion brain injury in the rat [290]. Interestingly, intravenous administration of pentoxifyline, a TNFα synthesis inhibitor, or of soluble TNFRs attenuates brain oedema formation and hippocampal neuronal death, and improves recovery of motor function [291]. Moreover, mice genetically deficient for TNF display less motor and cognitive impairment during the first week after traumatic brain injury, but are more severely affected later on and display increased post-traumatic mortality [292]. Similarly, p55 TNFR- and p75 TNFR-deficient mice have larger lesion size, greater number of apoptotic cells and worse recovery of motor function after spinal cord traumatic injury than wild-type mice [293]. Thus TNF produced by perivascular brain macrophages [213] and acting on TNFRs expressed by endothelial cells and neurons (see above) appears to mediate hippocampal neuronal death and motor and cognitive impairment in the first days after traumatic brain injury. However, at later time points, TNF appears to limit lesion size and favours recovery of motor function after brain trauma.

Alzheimer’s disease

Several studies have shown a relationship between IL-1α and IL-1β polymorphisms and the age of onset of Alzheimer’s disease, and the influence of IL-1β polymorphisms on the disease course [294–296]. Interestingly, individual differences in Aβ-induced ex vivo cytokine production in monocytes are, to a large extent, of genetic origin [297]. Moreover, the production of IL-1 is closely related to plaque distribution and evolution in Alzheimer’s disease [298]. As indicated above, experimental studies show that Tg2576 mice containing a transgene of the Swedish double-mutation of human APP, induces IL-1β expression in astrocytes around Aβ plaques of aged animals [299]. Conversely, IL-1β administration in the brain increases the expression of APP [300] and its secretion by vascular smooth muscle cells isolated from the meninges of aged dogs with cerebral amyloid angiopathy [301]. Interestingly, IL-1 also reduces intracellular Aβ accumulation in these cells [301], a phenomenon that can be explained by in vitro observations showing that IL-1β enhances γ-secretase activity resulting in increased production of Aβ [302]. In view of these findings, it seems likely that IL-1 and Aβ exert a mutual positive feedback on each other’s production. Recently, it has been shown that enhanced IL-1 signalling in IL-1ra-deficient mice is associated with increased mortality.
and more neuronal damage in the hippocampus after intracerebroventricular infusion of human Aβ [303]. Moreover, oral administration of a brain-penetrating cytokine synthesis inhibitor reduces ex vivo hippocampal IL-1 production and attenuates the reduction in hippocampal synaptic dysfunction and learning deficits provoked by intracerebroventricular infusion of human Aβ [304]. Taken together, these observations indicate that IL-1 produced by blood vessels in Alzheimer’s disease or by astrocytes in animal models and acting on its signaling receptor expressed by brain endothelial cells and perivascular macrophages or hippocampal neurons (see above) plays an important role in mediating neuronal damage.

The allele in the TNFα promoter that is associated with higher TNF production [305] is neither associated with a higher risk for Alzheimer’s disease nor does it influence CSF Aβ levels [306]. It is, however, associated with a lower age of onset of Alzheimer’s disease compared with non-carriers of this allele [307]. Although conflicting data exist, some studies have shown a relationship between other polymorphisms in the TNFα promoter and the risk for Alzheimer’s disease [306,308]. Interestingly, expression of MADD, a protein protecting against apoptosis and competing with TRADD for binding to the intracellular domain of the p55 TNFR, has been found to be reduced in brain homogenates from patients with Alzheimer’s disease and to be correlated with neuronal cell death in the hippocampus [309]. As with IL-1, TNF increases the secretion of APP by vascular smooth muscle cells isolated from the meninges of aged dogs with cerebral amyloid angiopathy and reduces intracellular Aβ accumulation [301]. Experimentally, it has been shown that intrahippocampal injection of TNFα along with an Aβ fragment impairs working memory [310]. Moreover, it has recently been shown that oral administration of a brain-penetrating cytokine synthesis inhibitor reduces ex vivo hippocampal TNF production and attenuates the reduction in hippocampal synaptic dysfunction and learning deficits provoked by intracerebroventricular infusion of human Aβ [304]. Collectively, these lines of evidence indicate that TNFα plays a role in Aβ production and in mediating neuronal death in Alzheimer’s disease. Whether TNFα produced by endothelial cells acts in the (peri)vascular compartments or directly on TNFRs expressed by hippocampal neurons remains unknown.

CONCLUSIONS

In the first part of this review, we have dealt with the organization of (peri)vascular compartments in the healthy CNS and with the changes occurring in these compartments during different types of pathology. The relevance of the (peri)vascular compartments to pathology appears, in some cases, to be even greater for infections or injuries affecting the brain than for similar types of insults in peripheral tissues. Several reasons can be put forward to explain this. First, the source of infection is exogenous for the skin, lungs and intestines, but blood-borne for the CNS which makes the brain (peri)vascular compartments more relevant to infection by pathogens than peripheral organs. Secondly, the maintenance of the blood–brain barrier by endothelial cells and of ionic gradients by astrocytes requires energy. Disruption of blood flow and the supply of energy substrates after ischaemic or traumatic brain injury results in blood–brain barrier breakdown and the failure of astrocytes to maintain ion gradients, thus leading to a loss of neuronal function. Thirdly, energy substrates are shunted from blood to neurons via perivascular astrocytes. As a consequence, in case of insults to the (peri)vascular brain compartments, the energy flow to neurons is disrupted, whereas in the periphery tissue glucose may continue to reach the underlying tissue through diffusion. Fourthly, active brain–to–blood transport mechanisms are the bottleneck in the clearance of molecules, such as Aβ, from the brain, as, unlike the fenestrated peripheral blood vessels, the blood–brain barrier does not allow passive diffusion into the bloodstream when tissue concentrations increase. The brain (peri)vascular compartments are thus prone to accumulation of molecules waiting to be cleared by rate-limited transport systems across the blood–brain barrier.

Perivascular macrophages are the most reactive (peri)vascular cell type after CNS infection and injury [311], and play important roles in initiating, mediating and propagating neuro-inflammatory processes. The CNS inflammatory responses mounted against infection or injury in tissues that lack a blood–brain barrier involve tissue oedema and pain, as in bacterial meningitis or traumatic brain injury, and are thus reminiscent of the responses in peripheral tissues. However, infection or injury of CNS tissues that are protected by a blood–brain barrier, such as in Alzheimer’s disease, does not result in tissue oedema and pain. As discussed in the present review, the production of the pro-inflammatory cytokines IL-1 and TNFα by perivascular macrophages is a common denominator in various neuropathologies, including bacterial meningitis, HIV-1-associated dementia, MS, ischaemic and traumatic brain injury, and Alzheimer’s disease. The roles of IL-1 and TNFα in neuropathology have been studied in some depth in both animal models and clinical situations of neuro-inflammation and -degeneration. The main target for IL-1 and TNF produced in the brain (peri)vascular compartment is the endothelium, where these cytokines induce the expression of adhesion molecules, such as ICAM-1 (intercellular adhesion molecule-1), that slow down circulating leucocytes and favour their extravasation. With respect to the cellular targets of pro-inflammatory cytokines, it is important to keep in mind that several studies have shown
that IL-1 and TNF promote astrogliosis [265,312] and glial production of neuronal growth factors when applied directly in the brain or onto cell cultures [313,314]. However, few studies have actually addressed the effects of these cytokines on astrocytes and the expression of growth factors in animal models relevant to the brain pathologies discussed in this review and this warrants further study. While awaiting these studies, it is clear that IL-1 and TNFα can act as a double-edged sword, i.e. mediating both detrimental and beneficial effects depending on the site of infection or injury, the local cytokine concentrations and the timing of cytokine production. In view of this, we feel that, although pro-inflammatory cytokine production and action in the (peri)vascular compartments of the CNS appear to be realistic targets for future intervention strategies to fight neuropathologies, this cannot proceed without a clear understanding of the role of IL-1 and TNF in both damaging and repair processes.

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