Key words: Nitric oxide synthase, Microglia, Astroglia, Transforming growth factor β, Plasminogen activators, Neurodegeneration

Introduction

The central nervous system (CNS) consists of two major cell types, neurones and glial cells. Neuronal communication with other neurones or glial cells is effected mainly through neurotransmitters and peptides, while glial cells appear to use an abundant range of factors for the communication with either neurones or other glial cells. The communication between neurones can span large distances in the body, while glial cell communication is mainly local or paracrine. During the past decade it has become clear that the glial cells named after glue (= glia) have more functions besides acting as a "nerve-glue" to form the brain. The glial cells appeared to be essential for neuronal protection, survival and outgrowth during development and for the neuronal degeneration and regeneration under pathological conditions. In this review we summarize glial cell functions and focus on the role, production and regulation of nitric oxide (NO), a molecule which has been shown to be involved in various neuroimmune processes.

Glial Cells

Glial cells can be divided into microglial cells and macroglial cells, the latter of which are subdivided in astroglial cells and oligodendrocytes. The oligodendrocytes are known for their myelin production that wraps the axons in the white matter of the brain, and are affected in diseases like multiple sclerosis (MS). Glial cell activation has been indicated in many neuropathological diseases like Alzheimer’s disease, Parkinson’s disease, multiple sclerosis, acquired immune deficiency syndrome (AIDS) dementia complex (ADC) and other disorders.

Microglial cells

Microglial cells, formerly named Hortegaglia or Mesoglia, have been described in detail for the first time in 1932 by Del Rio-Hortega considered as the father of microglia. Microglial cells are ubiquitously distributed in the CNS, show heterogeneous morphology and comprise up to 20% of the total glial cell population in the brain. It was Del Rio-Hortega who proposed that microglial cells occur in two morphologically distinct forms, the ameboid or macrophage-like form representing as active microglial cells seen in developing brain and at sites of injury. These cells convert into the highly branched ramified microglial cells viewed as quiescent cells in the mature CNS which eventually can transform into active macrophages (reactive microglial cells) (Fig. 1). Del Rio-Hortega suggested that microglial cells served as macrophages or phagocytic cells, which has found substantial support.

The name mesoglia was derived from their proposed mesodermal origin. Indeed more recent and elaborative studies support a bone-marrow origin from the usual macrophage precursor. Blood monocytes or monocyte precursors invade the brain during development. In studies using chimeric rats, support was found for the bone marrow origin hypothesis but in other rat chimera experiments the contrary was found. The prevailing concept is however that blood monocytes are the precursors of ameboid microglial cells. There is apparently no need...
for the influx of a large number of monocytes into the brain parenchyma under normal conditions, since microglial cells have an extreme long life and have a low turnover rate. Their numbers can be augmented by local proliferation or by immigration of blood monocytes. Under pathological conditions increased monocyte infiltration into the brain parenchyma is found and the influx is suggested to be mediated by the microglial cell derived chemokine MCP-1 or adhesion molecules.

Microglial cell functions
The microglial cells serve as immunoregulatory cells, and are essential for resistance to inter- and intracellular pathogens. The microglial cells are present in a resting or a ramified form (Fig. 2) and are very important in immunosurveillance. After activation of the resting cells the microglial cells exhibit a highly potent phagocytic activity of foreign organisms and material, phagocytosis of injured or necrotic tissue, antimicrobial immunity, elimination of tumour cells and regulate inflammatory responses. Microglial cells have been reported to possess Fc receptors, CD4 antigen and major histocompatibility complex (MHC) class I and II antigens and thus are antigen presenting cells, Interferon-γ and interleukin-4 (IL-4) up-regulate MHC class II expression and induce microglial cell proliferation. In addition, microglial cells show chemotaxic activity, like monocytes and macrophages, to several immunological factors such as complement factor C5a and to transforming growth factor β (TGFβ) suggesting that these cells can move to sites of injury and thereby participate in an inflammatory response. These observations have led to a redefinition of the brain as immune privileged site a concept based on lack of inflammatory responses through the absence of T and B cells.

Microglial cells share many functional characteristics with cells of the monocyte lineage. Interleukin-1 (IL-1) production by microglial cells has been demonstrated for the first time by Giulian, and later by many others and IL-1 is found to be present in injured brain tissue. Other cytokines produced by microglial cells are TNF-α, IL6, IL7–IL9 and TGFβ and they produce prostanoids such as prostaglandin E2, thromboxane and leukotrienes, LTC4, LTB4, 5-HETE. IL-5 was found to be produced by microglial cells in vitro which may be involved in the interaction between glial cells and immune cells in the brain. IL-10 and TGFβ both immunosuppressive and anti-inflammatory cytokines, have been demonstrated to be produced by human microglial cells and down-regulate microglial cell functions. These properties confirm that microglial cells can initiate and regulate immune and inflammatory responses within the brain.

Astroglial cells
Astrocytes, oligodendrocytes and neurons are of ectodermal origin, and derive from the neuroepithelium of the primitive neural tube. Astroglial cells, unlike neurons, retain the ability to divide throughout life. The multipotential stem cell develops into the bipotential progenitor cell and finally the glial lineage-restricted progenitor cell which can differentiate into the oligodendrocyte, the astrocyte type 1 and type 2. The astrocytes outnumber neurons 10:1 in mammalian brain, and as their name imply, they have a star-shaped morphology. The astrocytes can be identified by using specific markers such as glial fibrillary acidic protein (GFAP), and glutamine synthase (GS), that are specific for both types of astroglial cells.

Each cell forms processes that contact the blood vessels, where they form the so-called end-feet or sucker processes which also forms part of the blood–brain barrier (BBB) together with endothelial cells and the lamina basalis. Astrocytes in the white matter are referred to as fibrous astrocytes, with numerous fibrils within their cytoplasm. In the grey matter, the astrocytes generally contain few fibrils and are called protoplasmic astrocytes. Interestingly, in vitro also two types of astrocytes can be identified, type 1 and type 2 astrocytes which are thought to be in vivo analogous of the protoplasmic and fibrous astrocytes respectively (Fig. 2).

Astroglial cell functions
Initially the function of astroglial cells was thought to be a structural support within the CNS, with their processes having junctions with other astroglial cells, endothelial cells and neurons. In addi-
tion in repair mechanisms the astroglial cells fill open spaces by proliferating and thereby forming a glial scar.83–86

It is now known that astroglial cells are very important cells in the outgrowth and survival of neurones during development and in neuropathology. Astroglial cells produce nerve growth factor (NGF)87 in vitro and in vivo and the production of NGF is increased by IL-1,88–92 TNFα,93 IL-4 and IL-5.94 Other neurotrophic factors produced by astroglial cells are ciliary neurotrophic factor (CNTF), brain-derived neurotrophic factors (BDNF) and fibroblast growth factor (FGF).95,96

Astrocytes produce cytokines like IL-1,4,97–100 IL-6,101 IL-3,102 TGFβ103,104 and IL-15106 and factors like prostaglandin E2 (PGE2),97,99,107,108 granulocyte-macrophage colony-stimulating factor (GM-CSF)109,110 and microglial mitogens (MM).111 TNFα is also produced by astroglial cells in vitro after lipopolysaccharide (components of Gram-negative bacterial outer membranes; LPS)56,112 and IL-1β stimulation59,106 or mycoplasma infection.108

Astrocytes can be induced to express MHC I and II class molecules in vitro and are able to present antigen and thus are important immune cells in the brain. However, in contrast to microglial cells they do not express significant levels of MHC class II molecules in vivo.115

Glia–glia interactions

Recent studies have shown various interactions between microglial cells and astroglial cells. IL-1 produced by microglial cells has been shown to stimulate astroglial cell proliferation in vitro.116–119 Intracerebral injections of IL-1 or local production of IL-1 by microglial cells elicit astrogliosis which may result in scar formation120,121 and thereby have a negative effect on axonal outgrowth and remyelination. In addition the microglial cells influence the production of NGF by astroglial cells, which is enhanced after IL-1 and IL-5 both produced by microglial cells.66,94,122

On the other hand, astroglial cells influence microglial cell functions. Interleukin-3 (IL-3) is mitogenic to microglial cells and has been suggested to be produced by astrocytes.102,123 Mitotic activity of microglial cells can additionally be elevated by colony stimulating factor-1 (CSF-1), which production is increased by IL-1, TNFα,124 granulocyte-macrophage

FIG. 2. Microglial cells and astroglial cells in rat brain in vivo and in vitro stained for GSA-I-B4-isolectin and GFAP respectively. (A) GFAP staining of astroglial cells in rat brain; (B) GSA-I-B4-isolectin staining ramified microglial cells in a rat brain; (C) GFAP in vitro in a purified astroglial cell culture; (D) GSA-I-B4-isolectin in vitro in a purified ameboid microglial cell culture. A, B, bar = 66 mm; C,D, bar = 25 mm.
colony-stimulating factor (GM-CSF) or microglial mitogen (MM).

The microglial cells undergo morphological changes in response to factors released by or through cell–cell contact with astrocytes. These factors derived from astrocytes, induce microglial cells to become the ramified, functionally resting cells in vitro, while inflammatory mediators like interferon \( \gamma \) (IFN\( \gamma \)) and LPS induce microglial cells to become ameboid. Also, blood monocytes and spleen macrophages differentiate into ramified microglial cells when cultured onto an astroglial cell monolayer.

Glial–neurone interactions

The presence of glia-derived cytokines in the CNS and the function of these cytokines in vitro suggest that they are important for normal brain development and homeostasis. However, excessive expression of these cytokines may be a factor in abnormal glial functions leading to neuropathological events.

In general astrocytes have been found to express neurotrophic factors such as ciliary neurotrophic factor (CNTF), neurotrophin-3, fibroblast growth factor (FGF) and NGF near the site of injury. In vitro experiments show that astrocytes protect dopaminergic neurons against \( \text{H}_2\text{O}_2 \) toxicity through actions of glutamate. Interestingly, survival of these dopaminergic neurons in vitro is enhanced by the presence of glial cells derived from striatal astroglia: the target-derived astroglial cells, illustrating astroglial cell heterogeneity. The survival of dopaminergic neurons is promoted by glial cell-lined derived neurotrophic factor (GDNF) in vitro. In vitro cocultures of neurons and astrocytes induced an increased cell survival and neurite outgrowth and axons might trigger glial differentiation.

Neurones are less sensitive to oxygen or glucose deprivation or treatment with glutamate when co-cultured with astrocytes. In addition astrocytes increase neuronal survival under pathological conditions since they have an energy reserve stored as glycogen which becomes available for neurones under conditions of energy substrate limitations.

Astroglial cells are important in the metabolism of glutamate and GABA and other neurotransmitters, and maintain the microenvironment by regulating the ionic composition of the extracellular space around the neurones.

Neurotrophic effects i.e. neuronal survival and neurite extension have also been reported by microglial cell conditioned medium and more specifically by the production of NGF and thrombopoindin. In addition secretion of IL-6, IL-1, FGF, TGF\( \beta \), TNF\( \alpha \) by microglial cells and astroglial cells may stimulate nerve growth factor production by astroglial cells for the regeneration of neurones. These factors also directly improve the survival of neurones and/or have synergistic effects with NGF.

While in general factors released by astroglial cells actually increase the survival of neurones, microglial cells in contrast, can directly participate in neuronal cell death through the release of neurotoxins.

Microglial cells have been reported in the presence of degenerating neurones in various regions in the brain. In these regions the microglial cells clearly contribute to the removal of pycnotic cell bodies. Prior to this scavenger role, the active participation of microglial cells in neurite amputation has been shown on electron microscopic pictures of microglial cells engulfing axon processes which display no obvious signs of degeneration. It is therefore thought that interactions between neurones and microglial cells may not be restricted to cell debris scavenging but microglial cells may also induce neuronal cell death.

The release of these excitatory amino acids points to a further role of microglial cells in NMDA receptor-mediated neuronal injury. The release of these excitatory amino acids points to a further role of microglial cells in NMDA receptor-mediated neuronal injury. In addition, cultured microglial cell release large amounts of \( \text{H}_2\text{O}_2 \), which leads to neuronal cell death in neurone–microglial cell cocultures.

Taken together, activated microglial cells display a broad repertoire of cytotoxic functions which could be involved in tissue damage during CNS injury. In addition, microglial cells activate astroglial cells in a way that benefits regeneration. Further, the effect of neurotoxic factors released by microglial cells can be
attenuated by proteins released from astroglial cells.\textsuperscript{178,179} This illustrates the fascinating and delicate interactions that exist between glial cells and neurones, which are crucial in maintaining neural functioning and integrity. These studies have contributed towards a concept which considers reactive microglial cells as an opposing force to neurotrophic astrogia, the two glial cell populations rivaling in regulating survival of neurones.\textsuperscript{180}

**Nitric Oxide in the CNS**

Various cell types in the CNS, i.e. neurones, endothelial cells, microglial cells and astroglial cells produce NO. Different isoforms of the nitric oxide synthase (NOS) are responsible for the production of NO by these cell types. NO in the brain is multipotent and is responsible for blood flow regulation, may act as a neurotransmitter or as a neurotoxic agent, depending on the cellular source, amount and production site.

**Isoforms of nitric oxide synthase**

NO, a free radical gas, was found to be responsible for the vasodilatation in arteries and at first named endothelium derived relaxing factor (EDRF).\textsuperscript{181} Later the source of NO was elucidated revealing the enzymatically conversion of L-arginine to L-citrulline by NO synthase whereby NO is produced (Fig. 3).\textsuperscript{182} Three isoforms of NOS, encoded by different genes\textsuperscript{183} have been characterized, isolated and cloned to further study the physiologic and/or pathologic functions of NO.\textsuperscript{184} All three isoforms, endothelial (eNOS, ecNOS or type III), constitutive (cNOS, nNOS, bNOS or type 1) found in astroglial cells and neurones and inducible (iNOS, mNOS, macNOS or type II), NOS are found in the CNS and play a role in certain physiological or pathological functions of the CNS (see following section).\textsuperscript{185} Constitutive NOS is constitutively expressed in neurones, is Ca\textsuperscript{2+} and calmodulin dependent, and mediates the production of only small amounts of NO after stimulation. This neuron derived NO is very rapidly produced and released since cNOS is constitutively expressed and does not require mRNA synthesis, acts as a neurotransmitter with properties that differ from other neurotransmitters: (a) it is not stored in vesicles, (b) there are no specific release or uptake mechanisms and (c) its transmission is not synaptic. NO diffuses into the target cell and directly regulates enzymes systems, such as activation of guanylate cyclase, resulting in increased cGMP levels.\textsuperscript{186,187} NO has a half life of a few seconds in contrast to the milliseconds of the neurotransmitters in classical synapses.\textsuperscript{188,189} It is important in neurotransmission, and NO is considered a candidate for a memory-related process named long-term-potentiation (LTP).\textsuperscript{190,191} Indeed, inhibitors of NOS can block LTP.\textsuperscript{192,193} In areas such as the cerebral cortex, hippocampus, cerebellum and corpus striatum, cNOS expressing neurones compose 1–2\% of all neuronal cells.\textsuperscript{194} Activation of cNOS in astroglial cells was observed after challenge with calcium ionophores, bradykinin or glutamate.

Endothelial NOS (eNOS) produced by endothelial cells is a constitutive Ca\textsuperscript{2+}-dependent enzyme that is essential for the control of vascular tone. NO transduces a signal from the endothelial cell to the vascular smooth muscle eventually leading to cGMP production and vasodilation. In the brain, eNOS-derived NO regulates cerebrovascular blood flow.\textsuperscript{195}

Inducible NOS (iNOS) is a Ca\textsuperscript{2+} and calmodulin independent enzyme. It requires gene transcription, is slowly produced and is activated only under pathological situations where microglial cells and macrophages exert cytotoxic effects in response to cytokines.\textsuperscript{196} The generation of NO by iNOS is long-lasting, in contrast to the cNOS and eNOS isoforms where NO is generated in short bursts.\textsuperscript{197,198} The mechanism of iNOS induction involves transcription of mRNA and novel protein synthesis and it takes several hours before NO is generated after the initiating signal.\textsuperscript{199} It induces a 100-fold higher local concentrations of NO than eNOS or cNOS and act as a antimicrobial defence mechanism of the immune system. iNOS is not expressed in normal brains but expression can be induced in astroglial and microglial cells through viral infection or trauma.\textsuperscript{200,201} It is mainly expressed under inflammatory conditions, and after transient ischaemic periods.\textsuperscript{202–205}

**Nitric oxide in neuropathology**

NO can be neurotoxic under different circumstances. The NO mediated neuronal cell death can be induced by overexpression of cNOS in neurones and astroglial cells or iNOS induction in glial cells, both pathways will be further discussed below (see Fig. 4).

\textbf{cNOS induced NO mediated neurotoxicity}

Glutamate binding to its NMDA receptor induces NO production by cNOS activation. Derangements of
glutamate neurotransmission leading to neurotoxicity has been implicated in Alzheimer's disease, Huntington's disease, amyotrophic lateral sclerosis (ALS), epilepsy and stroke.206,207 Glutamate neurotoxicity is demonstrated to be mediated through NO production. After binding of glutamate with the NMDA subtype of glutamate receptors, Ca$^{2+}$ enters the channel and binds to calmodulin, a cofactor for cNOS and stimulates NOS activity whereby NO is produced.208 In addition, O$_2^-$ is produced which results in the formation of ONOO$^-$ which subsequently leads to neuronal death.209–211 Neurons obtained from cNOS null transgenic mice are markedly resistant to ischaemic conditions,212 in which the primary mechanism of damage is mediated by activation of the NMDA receptor and subsequent formation of NO. This indicates that cNOS is capable of producing neurotoxic amounts of NO.213

iNOS induced NO mediated neurotoxicity

The iNOS-mediated release of NO by astrocytes and microglial cells in the brain may be important in antimicrobial or tumoricial responses to inflammatory signals.197 In acute CNS inflammatory conditions like rabies, herpes simplex, Borna, and lymphocytic choriomeningitis virus, iNOS is expressed200,214–217 as well as in experimental pneumococcal meningitis and toxoplasmosis and in humans during encephalitis.130,218–220 iNOS mediated NO is considered to mediate neuronal and oligodendrocyte degeneration under neuropathological conditions.202,221,222 The role of iNOS in neuropathology in general is indirect, microglial cells and/or macrophages become activated either through direct infection with virus or other pathogens, or by local cytokine production and subsequently produce iNOS which eventually leads to damage. Excessive amounts of NO produced by glial cell are probably neurotoxic. iNOS-derived NO is one of the major sources of toxic free radicals in the brain, since its reaction with the superoxide anion (O$_2^-$) leads to the formation of peroxynitrite anion (ONOO$^-$) which is an extremely potent oxidizing agent.223 Peroxynitrite generates DNA-single-strand breaks with subsequent activation of the DNA repair enzyme poly ADP ribosyltransferase (PARS).224 Furthermore, NO and peroxynitrite have been shown to inhibit the mitochondrial respiratory chain and disrupt normal cellular iron homeostasis.201,225–227 Peroxynitrite and/or NO can terminally damage neurons, leading to cell death.228–232 Low levels of or sustained exposure to NO or peroxynitrite cause apoptosis, whereas sudden exposure to high concentrations of NO or peroxynitrite leads to necrosis.233

NO in Alzheimer's disease and MS

iNOS expression is found in Alzheimer's disease234,235 and in experimentally infected brains of rats with various viral agents.200 NO has been implicated in demyelination and destruction of oligodendrocytes and subsequent demyelination, the process found in MS236 and in the primary animal model for MS, experimental allergic encephalomyelitis (EAE).216,237 During EAE, iNOS mRNA is detectable before the onset of the clinical symptoms and the levels of protein correlate with the severity of the disease.200 Further evidence supporting a role for iNOS in the pathogenesis of MS is the finding that human iNOS protein and mRNA is markedly elevated in the active lesions in brains of MS patients.238,239 In MS lesions, macrophages appear to produce iNOS and are NADPH-diaphorase positive after histochemical staining.240 In another study, NADPH-diaphorase activity in MS lesions was found in reactive astroglial cells239 which was later shown to be cNOS.240 The cellular source of iNOS mRNA expression in brains of patients with MS has been confirmed to be macrophages/microglial cells.236 In EAE an increase was found in cNOS in blood vessels in the inflamed lesions and increase in iNOS in infiltrating inflammatory cells.216,241 iNOS expression can be (further) induced by cytokines like TNF$\alpha$, IFN$\gamma$ and IL-1, that are all detected in brains of MS patients.242–245

NO in AIDS dementia complex

iNOS-mediated NO production has also been described to be involved in acquired immune deficiency syndrome (AIDS)-related neuropathy. iNOS protein and mRNA was found in the CNS of patients with AIDS dementia complex (ADC),246,247 and is
expressed at higher levels than in brains of AIDS patients without neurological symptoms. The cell types expressing iNOS however, remain unknown. In these patients there is a clear correlation between the severity of the dementia and iNOS expression in the brain. In addition in brain tissue of simian immunodeficiency virus (SIV) infected monkeys iNOS mRNA was detected.248 The microglial cells have been postulated to be involved in the pathogenesis of ADC because they are preferentially infected by the virus.43,249–251 Neuropathological manifestations such as loss of cortical neurones, loss of synapses and neuronal apoptosis have therefore been suggested to be mediated indirectly by cytokines like TNFα252,253 and IL-1254–256 or by nitric oxide.257–261 TNFα, IL-1 and iNOS have been demonstrated in brains of AIDS patients247,256,262–265 as well as in ADC patients259,266,267 (Vincent et al., submitted). In cytomegalovirus (CMV) infected retinas from AIDS patients iNOS immunoreactivity and NADPH-diaphorase were found in (CMV)-infected cells identified as Müller cells and astrocytes.268

**NO in parkinson’s disease**

In Parkinson’s disease, which is primarily characterized by a loss of midbrain dopaminergic neurones, iNOS was present in glial cells in the mesencephalon probably in activated macrophages.63 In addition, NOS inhibitors attenuate malonate-induced degeneration by NMDA receptor activation of the nigrostriatal pathway in rats270 suggesting a role for NO in neurodegeneration. The inhibition of tyrosine hydroxylase resulting in reduced dopamine synthesis is triggered by peroxynitrite, a reaction product of NO.

**NO in brain activation**

During ischaemic brain damage eNOS is induced in endothelial cells which then has beneficial effects by enhancing the vasodilatation, further increasing blood flow in the peri-infarct area.272,273 In addition iNOS is induced leading to NO production, which leads to neuronal death after cerebral ischaemia.274 During postnatal brain development of rats, large numbers of NADPH-diaphorase positive neurones and NADPH-diaphorase positive cells with macrophage morphology were observed. The latter are possibly involved in developmental shaping of the brain, which includes cell death and fagocytosis of cellular debris.275 High levels of NADPH-diaphorase were found in evenly distributed astroglial cells in areas surrounding a mechanical lesion in the brain. Within the lesion the NADPH-diaphorase positive cells most probably were macrophages.276 Thus, although iNOS and NADPH-diaphorase activity can be induced in astrocytes and microglial cells through viral infection or trauma most of the studies have revealed iNOS immunoreactivity and iNOS mRNA in the brain in infiltrating macrophages or microglial cells.210 In general, exposure of brain cells to signals, such as microbial products, viruses, glumatate or yet unknown signals in diseases like MS, Alzheimer’s and Parkinson’s disease leads to the secretion of inflammatory cytokines that induce either de novo synthesis of iNOS by glial cells or cNOS in astroglial cells or neurons as has been demonstrated in in vitro studies. This NO may be neurotoxic and may subsequently lead to neuropathology.

**Production of iNOS in vitro**

To answer some more fundamental questions regarding iNOS production and regulation in vitro studies are widely used. Sources, induction mechanism and intervention of iNOS production are studied in detail in various glial cell cultures. In addition, a model has been developed that allows studies of the possible neurotoxic effects of glial NO, on cultured neurons. These in vitro studies have led to new insights in the functions of glial cells in normal brain and in neuropathology.

Several techniques have been described for isolation of rat murine or human brain microglial cells, astroglial cells and maintenance of these cells in vitro.118,240,277–282 The microglial cells and astroglial cells are isolated from mixed glial cultures in most instances or from disrupted adult or neonatal tissue.53,127,171,279,283–285 In tissue culture, the ameboid and ramified microglial cell can be identified, most probably corresponding to its morphological diversity in the adult brain.286 The astroglial cell cultures can be contaminated with microglial cells, since the purification of astroglial cells is a rather delicate technique and microglial cells remain a significant contaminant.56,161,287 Many studies showing production of, for example, cytokines like IL-1 or TNFα by astroglial cells in vitro have to be interpreted with care. In vitro studies also provide a very useful technique to study both the functions of glial cells and of neurones as well as the interactions between these cell types. Therefore techniques for selective isolation, co-culturing, labeling and stimulation of microglial cells and astroglial cells allow investigators to study some fundamental questions in neuro-immunology.

**Glial cell-derived iNOS**

iNOS expression by glial cells has been studied in in vitro systems of highly purified microglial cell and astroglial cell cultures and in mixed cultures containing both cell types from human or rodent brain. Following incubations with various stimuli, both microglial and astroglial cells have been demonstrated to produce nitrate, one of the end-products of nitric oxide oxidation.203
iNOS has been identified in rodent astrocytes and microglial cells in response to IL-1β, LPS or Gram-positive bacterial products. IFNγ induces NO in microglial cells and macrophages but not in astroglial cells, while synergism of IFNγ and IL-1β or TNFα induce significant levels of nitrite in rodent and human astroglial cells. LPS induction of iNOS required CD14 expression on glial cells. As yet it is not clear whether cytokines activate gene expression via one or multiple pathways. Experiments with phorbol esters, which induce iNOS, suggest that protein kinase C may be involved in the induction process in microglial cells and astrocytes.

Agents like IFNγ and LPS are more effective inducers of iNOS in rodent than in human microglial cells, which therefore appears to be species-dependent as described for NO production by retinal pigment epithelial cells. Recently, some studies did however reveal NO and iNOS mRNA production in human ramified microglial cells upon LPS or TNFα stimulation.

HIV or the HIV type I coat proteins gp120 or gp41 induce iNOS in cultured microglial cells, monocytes or macrophages. In human fetal glial cells, comparable amounts of NO were induced by gp120, gp41 and the proinflammatory cytokines IFNγ and IL-1β. HIV-infected brain mononuclear macrophage secrete NO and O2− especially after immune activation and TNFα further increases NO production. In MS and EAE, macrophages isolated from lesion areas produced significant amounts of nitrite and were shown to be iNOS positive without any further stimulation.

β-amyloid, the major component of the senile plaques in Alzheimer's disease, causes a significant increase in NO by microglial cells and not in astroglial cells. The NO production induced by the β-amyloid was increased by IFNγ or phorbol-myristate-acetate (PMA) challenge.

Studies that have attempted to compare rat microglial cell and astrocyte NO production have concluded that microglial cells produce more NO on a per cell basis than astrocytes. This has led to the suggestion that activated microglial cells rather than astrocytes are the principal source of reactive nitrogen intermediates in the CNS, and that the NO produced by astroglial cells might be beneficial, whereas microglial-derived NO might be involved in neurotoxicity.

Nitric oxide mediated neurotoxicity in vitro

In vitro co-cultures of glial cells with neurones have proven to be a valuable tool in the identification of NO as a neurotoxin and the cellular sources of NO. Exogenous NO generated from NO donors have been shown to kill neurones in vitro. Cytokine-activated murine microglial cells and astroglial cells apparently generate substantial amounts of NO that kill neurones since inhibition of endogenously formed NO by specific NOS inhibitors blocks this microglia- or astrocyte-mediated neurotoxicity. For example, factors like LPS, gp41 and β-amyloid can indirectly kill neurones in mixed cultures with glial cells, which is abrogated by NOS-inhibitors. LPS or cytokine activated glial cells stimulate the production of neurotoxins, e.g. NO, because LPS and cytokines do not directly influence the viability of purified neurones.

The glial cells produce large amounts of NO by iNOS activity which can form peroxynitrite which is toxic to neurones as previously described. In addition, glutamate neurotoxicity is also mediated by NO in primary neuronal cultures. Peroxynitrite, NO and NMDA can damage neurones in vitro leading to necrotic or apoptotic cell death pending on the concentration and duration of the exposure. In addition, oligodendrocytes are also being killed by an NO dependent mechanism by ameboid microglial cells in vitro, suggesting that iNOS expression by invading and intrinsic brain cells play a role in lesion formation in multiple sclerosis.

Although glial cells produce various neurotoxins i.e. TNFα, glutamate and PAF, production of NO appears to play a key role in different neurotoxic pathways since inhibition of iNOS spares the neurones. These in vitro findings suggest an important role for glial iNOS derived NO in the pathophysiology of CNS diseases.

Regulation of NO Production

Since NO is shown to play an important role in neurotoxicity, inhibition of NO could be a possible route of intervention in the prevention of neuropathogenesis. Experimentally used inhibitors of NOS production are synthetic arginine analogues like N(G)-mono-methylarginine (NMMA), Nw-nitro-L-arginine methyl ester (L-NAME), aminoguanidine and N(G)-nitroarginine. These studies clearly illustrate the indirect mechanism by which neurones are thought to be killed.

The specificity of these inhibitors for the subtypes of NOS, and thereby effects on eNOS and cNOS leading to e.g. vascular changes, may explain these different results. The search for pharmacological tools that selectively inhibit iNOS, eNOS or cNOS is currently getting much attention and thus far has yielded some agents. The
Transforming growth factor β (TGFβ)

TGFβ is a 25 kDa homodimemic protein secreted by a variety of cells as a latent protein complex. There are at least five distinct gene products that constitute the TGFβ family, TGFβ1 through TGFβ3 which show a high degree (70–80%) of amino acid sequence identity. The three highly homologous mammalian TGFβ isoforms are TGFβ1, TGFβ2 and TGFβ3 with relatively high sequence similarities but differences in receptor binding affinities. Three different TGFβ receptors have been identified, type I to type III, which distributions are ubiquitous in various body tissues.

In a variety of studies of microglial cell cultures, TGFβ has been shown to be a potent immunosuppressive cytokine and inhibits NO production by microglial cells. TGFβ inhibits iNOS expression by decreasing iNOS mRNA stability and inhibiting its translation and increasing iNOS protein degradation, resulting in reduced NO production. Not only NO but also O2− is inhibited by TGFβ and thereby the formation of the highly toxic peroxynitrite is prevented.

TGFβ is a chemotactic agent for monocytes and macrophages and is suggested to be important in the recruitment of circulating monocytes into brain tissue after damage. TGFβ has protective effects in different experimental autoimmune diseases whereas neutralizing antibodies to TGFβ worsen clinical severity. During EAE, and after cerebral trauma or hypoxic-ischaemic damage, TGFβ expression is increased when neurological symptoms are severe, which might indicate an inflammation limiting of TGFβ in the recovery phase thereby controlling the inflammatory reaction.

Astroglial and microglial cells are known to constitutively produce TGFβ in vivo and in vitro. The isoforms TGFβ1, TGFβ2 and TGFβ3 are produced by astroglial cells in vivo, while microglial cells only produce the TGFβ1 isoform. TGFβ production in microglial and astroglial cells is increased after TNFα or IL-1β or TGFβ exposure itself.

In co-cultures of astroglial cells and microglial cells, TGFβ was found to inhibit iNOS expression and thereby NO production by endotoxin activated microglial cells. The presence of astroglial cells was shown to be essential for the activation of TGFβ in these co-cultures of astroglial and microglial cells (see Fig. 5).

Regulation of TGFβ activity

TGFβ is produced by glial cells in a latent, inactive form and forms a complex with the latency-associated protein (LAP). Activation of latent TGFβ consists of releasing TGFβ from the LAP which occurs after heat treatment, acidification, alkalization or proteolysis by plasmin. Plasmin is generated by the plasminogen activator system. Plasminogen activators (PA) are serine proteases consisting of a 50 kDa urokinase-type plasminogen activator (u-PA)
and a 68 kD tissue-type plasminogen activator (tPA), secreted as inactive pro-forms, which major substrate is plasminogen. uPA and tPA can cleave plasminogen into plasmin which subsequently activates latent TGFβ354,355 (Fig. 6). These PAs are specifically inhibited by the plasminogen activator inhibitors (PAI), PAI-1, PAI-2 and PAI-3 (Table 1),356,357 by formation of a tight complex with PAs. Plasmin has a broad range of substrates including fibrin, fibronectin, laminin and matrix metalloproteinases (MMP). The PAs and PAIs play an important role by fine regulating the proteolytic degradation of fibrin clots (fibrinolysis) in the circulation, mediated by plasmin354,358 and therefore tPA is now used for the treatment of thrombotic stroke. Only recently functions for PA were found in the brain, and proteolysis of the extracellular matrix (ECM) by MMP activation, amplified by PAs, has been suggested. The breakdown of the ECM is thought to be involved in brain development and neurite outgrowth but also in neuropathology like growth and invasion of brain tumours, leukocyte infiltration in MS and EAE, breakdown of the BBB and nerve demyelination.311

Microglial cells secrete proteases such as elastase, uPA and secrete plasminogen359,360 which have a direct neurotrophic effects on various types of neurons.361 Astroglial cells can synthesize and secrete both tPA and uPA as well as PAI-1,362–364 and tPA is involved in motor learning but also in Alzheimer’s disease and neuronal degeneration.365–367 PAs and PAIs have been demonstrated in cerebrospinal fluid of patients with neurological disease368,369 and tPA expression was found370 in MS lesions supporting a role of PA and PAI in neuropathological processes.

An important role of tPA and PAI and the regulation of TGFβ activity was shown in a glial cell coculture.178 tPA and PAI-1 produced by astroglial cells regulated the bioactivity of TGFβ, and thereby indirectly the production of NO by microglial cells.178 Therefore, we postulate that tPA-mediated activation of TGFβ plays an important role in neuroprotection.

### Summary

In neuropathological conditions such as Alzheimer’s disease, Parkinson’s disease, AIDS dementia complex and multiple sclerosis, activation of microglial cells and astroglial cells is evident. Under these neuropathological conditions cellular damage in the brain is considered to arise indirectly from cytotoxic substances produced by activated glial cells. One of these toxins is NO which has been demonstrated to be produced during several neuropathological conditions. High NO levels are produced by glial cells and exert neurotoxic effects. Astroglial cells and microglial cells communicate in various ways to reduce NO production by microglial cells which is essential to maintain homeostasis in the brain. The production of TGFβ by glial cells and its activation by astrocyte-derived tPA represents one mechanism by which astroglia limit NO production in the brain.

### References

1. Virchow R. Ueber das Granulierte ansehen der Wandungen der Gehirnventrikel. Allg Z Psychiatrie 1846; 3: 242–250.
2. Somjen GG. Nervenkitt: notes on the history of the concept of neuroglia. GLIA 1988; 1: 2–9.
3. Itagaki S, McGeer PL, Akiyama H, Zhu S, Selkoe D. Relationship of microglia and astrocytes to amyloid deposits of Alzheimer’s disease. J Neuroimmunol 1989; 24: 173–182.
4. McGeer PL, Akiyama H, Itagaki S, McGeer EG. Immune system response in Alzheimer's disease. Gen Neurosci 1989; 15: 519–525.
5. McGeer PL, Itagaki S, McGeer EG. Reactive microglia are positive for HLA-DR in the substantia nigra of Parkinson's and Alzheimer's disease brains. Neurology 1988; 38: 1285–1291.
6. McGeer PL, Itagaki S, Tago H, McGeer EG. Reactive microglia in patients with senile dementia of the Alzheimer type are positive for the histocompatibility glycoprotein HLA-DR. Neurology 1987; 37: 195–200.
7. Rozemuller JM, Eikelenboom P, Bals ST, Stamm FG. Microglial cells around amyloid plaques in Alzheimer's disease express leucocyte adhesion molecules of the LFA-1 family. Neurology 1989; 101: 288–292.
8. Woodroffe MN, Bellamy AS, Feldman M, Davison AN, Gurner ML. Immunocytochemical characterization of the immune reaction in the central nervous system in multiple sclerosis. Possible role for microglia in lesion growth. J Neuropathol Exp Neurol 1986; 45: 747–765.
9. Koka P, He K, Zack JA, Kitchen S, Peacock W, Fried I, et al. Human immunodeficiency virus type 1 envelope proteins induce interleukin 1, tumor necrosis factor alpha, and nitric oxide in glial cultures derived from fetal, neonatal, and adult human brain. J Exp Med 1995; 182: 941–952.

10. Dixick DW, Lee SC, Hatch W, et al. Macrophages and microglia in HIV-related CNS neuropathology. In: Price RW, Perry SW, eds. HIV, AIDS and the Brain. New York: Raven Press, 1994; 99–118.
11. Giulian D, Yu J, Li X, Tom D, Li J, Wendt E, et al. Study of receptor-mediated neurotoxins released by HIV-infected mononuclear phagocytes found in human brain. J Neurosci 1996; 16: 3139–3153.
12. Río-Herrero D, Torres-Villafañe P. Pipeline and Cellular Pathology of the Nervous System, Vol. 2. New York: Hoeber, 1932; 481–534.
13. Lawson LJ, Perry VH, Dri P, Gordon S. Heterogeneity in the development and morphology of microglia in the normal, adult mouse brain. Neuroscience 1993; 39: 151–170.
14. Murabe Y, Sano Y. Morphological studies on neuroglia. Cell Tissue Res 21: 469–485.
15. Ling E, Wong W. The origin and nature of ramified and amoeboid microglia: A historical review and current concepts. Prog Clin Biol Res 1984; 15: 39–70.
16. Boya J, Calvo JL, Carbonell AL, Borregon A. A lectin histochemistry study on the development of rat microglial cells. J Anat 1981; 175: 229–236.
17. Boya J, Calvo JL, Carbonell AL, Borregon A. A lectin histochemistry study on the development of rat microglial cells. J Anat 1981; 175: 229–236.
18. Perry VH, Hume DA, Gordon S. Immunohistochemical localization of macrophages and microglia in the adult and developing mouse brain. Neuroscience 1985; 15: 313–326.
19. Marty S, Dusart I, Peschanski M. Glial changes following an excitotoxic lesion in the CNS-Microglia macrophages. Neuroscience 1995; 45: 529–539.
20. Leong S, Ling E, Amoeboid and ramified microglia: their interrelation-ship and response to brain injury. GLIA 1992; 6: 39–47.
21. Killackey HP. Glia and the elimination of transient cortical projections. J Anat 1981; 150: 39–47.
22. Killackey HP. Glia and the elimination of transient cortical projections. J Anat 1981; 150: 39–47.
23. Killackey HP. Glia and the elimination of transient cortical projections. J Anat 1981; 150: 39–47.
24. Killackey HP. Glia and the elimination of transient cortical projections. J Anat 1981; 150: 39–47.
25. Killackey HP. Glia and the elimination of transient cortical projections. J Anat 1981; 150: 39–47.
26. Killackey HP. Glia and the elimination of transient cortical projections. J Anat 1981; 150: 39–47.
27. Killackey HP. Glia and the elimination of transient cortical projections. J Anat 1981; 150: 39–47.
28. Killackey HP. Glia and the elimination of transient cortical projections. J Anat 1981; 150: 39–47.
29. Killackey HP. Glia and the elimination of transient cortical projections. J Anat 1981; 150: 39–47.
30. Killackey HP. Glia and the elimination of transient cortical projections. J Anat 1981; 150: 39–47.
31. Killackey HP. Glia and the elimination of transient cortical projections. J Anat 1981; 150: 39–47.
V. A. M. Vincent et al.

to inhibit macrophage cytotoxic activity. J Immunol 1992; 148: 3578–3582.

Hsu S, Sheng WS, Peterson PK, Chao CC. Cytokine modulation of murine microglial cell supernatant products. GLIA 1995; 15: 45–56.

Raff MC, Abney ER, Miller RH. Two glial cell lineages diverge prenatally in the rat optic nerve. J Neurosci Res 1991; 148: 137–146.

Matsuzawa T, Mizutani Y, Osawa T. Ontogenic of radial and other astroglial cells in murine cerebral cortex. GLIA 1991; 4: 138–148.

Goldman JE, Vaysse PJ. Tracing glial cell lineages in the mammalian forebrain. GLIA 1991; 4: 149–156.

Noble M. Production of cytokines in the 0–2A lineage: clocks and type-2 astrocytes. GLIA 1991; 4: 157–164.

Martin PM, O'Callaghan JP. A direct comparison of GFAP immunocytochemistry and GFAP concentration in various regions of ethanol-fixed rat and mouse brain. J Neurosci Meth 1995; 58: 181–192.

Eng LE, DaArmond SJ. Immunocytochemical studies of astrocytes in normal development and disease. Adv Cell Neurobiol 1982; 3: 145–171.

Akimoto J, Ishi H, Miwa T, Ikeda K. Immunohistochemical study of glutamine synthetase expression in early glial development. Dev Brain Res 1993; 72: 9–14.

Caldini M, Rolland B, Fages C, Tardy M. Glutamine synthetase activity during mouse brain development. Biochem Biophys Res Comm 1987; 187–196.

Dell'Anna ME, Geloso MC, Draisci G, Luthman J. Transient changes in Fos and GFAP immunoreactivity precede neuronal loss in the rat hippocampus following neonatal anoxia. J Neurochem 1991; 58: 125–133.

Miller RH, Raff MC. Fibrous and protoplasmic astrocytes are biochemically and developmentally distinct. J Neurosci 1984; 15: 187–192.

Ogawa M, Araki M, Nagatsu I, Yoshida M. Astroglial cell activation caused by neurotransmitters: immunohistochemical observations with antibodies to glial fibrillary acidic protein, laminin, and tyrosine hydroxylase. Exp Neurol 1989; 106: 187–196.

Dell'Anna ME, Geloso MC, Drasice G, Ludwig H. Transient changes in mouse brain development. Experientia 1982; 38: 1199–1202.

Sheehan SH, Lasek DJ, Jones JR, Snouffer SN, Stafford CA. Preferential histochemical staining of protoplasmic and fibrous astrocytes in rat CNS with GFAP antibodies using different fixatives. Brain Res 1990; 518: 347–352.

Giulian D, Vaca K, Johnson B. Secreted peptides as regulators of neuron-glia and glia-glia interactions in the developing nervous system. J Neurosci Res 1995; 60: 1374–1382.

Giulian D, Johnson B, Krebs JE, George JK, Tappert M. Microglial mitogens are produced in the developing and injured mammalian brain. J Cell Biol 1991; 112: 323–333.

Lafortune L, Nalbantoglu J, Antel JP. Expression of tumor necrosis factor-alpha (TNF alpha) and interleukin 6 (IL-6) mRNA in adult human astrocytes: comparison with adult microglial and fetal astrocytes. J Neurochem 1996; 65: 515–521.

Aloisi F, Borsellino G, Samoggia P, Testa U, Chelucci C, Russo G, et al. Astrocyte cultures from human embryonic brain: characterization and modulation of surface molecules by inflammatory cytokines. J Neuroimmunol 1992; 39: 307–318.

Aloisi F, Borsellino G, Samoggia P, Testa U, Chelucci C, Russo G, et al. Astrocyte cultures from human embryonic brain: characterization and modulation of surface molecules by inflammatory cytokines. J Neuroimmunol 1992; 39: 307–318.
120. Giulian D, Lachman LB. Interleukin-1 stimulation of astroglial proliferation after brain injury. *Science* 1985; 228: 497–499.

121. Berkenbosch E. Macrophages and astroglial interactions in repair to brain injury. *Ann NY Acad Sci* 1992; 650: 186–190.

122. Giulian D, Li J, Li X, George J, Rutecki PA. The impact of microglia-derived cytokines upon glialin in the CNS. *Dev Neurosci* 1994; 16: 128–136.

123. Frei K, Bodmer S, Schwerdel C, Fontana A. Astrocyte-derived interleukin 3 as a growth factor for microglial cells and peritoneal macrophages. *J Immunol* 1986; 137: 5521–5527.

124. Therby C, Stanley ER, Mallat M. Interleukin 1 and tumor necrosis factor-alpha stimulate the production of colony-stimulating factor 1 by murine astrocytes. *J Neurochem* 1992; 59: 1183–1186.

125. Liu W, Brosnan CF, Dickson DW, Lee SC. Macrophage colony-stimulating factor mediates astrocyte-induced microglial ramification in human fetal central nervous system culture. *J Neurochem* 1990; 54: 454–460.

126. Schmidmayer J, Jacobsen C, Mikkgs C, Sievers J. Blood monocytes and spleen macrophages differentiate into microglia-like cells on monolayers of astrocytes: morphology. *GLIA* 1994; 12: 254–258.

127. Griffin WST, Stanley LC, Ling C, White L, MacLeod V, Perrot LJ, et al. Brain-interleukin-1 and S100 immunoreactivity are elevated in Down syndrome and Alzheimer disease. *Proc Natl Acad Sci USA* 1989; 86: 7611–7616.

128. Hertz L. Neuronal-astrocytic interactions in brain development, brain function and brain disease. *Adv Exp Med Biol* 1991; 296: 143–159.

129. Brenneman DE, Schultzberg M, Bukker MM, Hirschcock ER. Direct interaction with target-derived glia enhances survival but not differentiation of human fetal mesencephalic dopaminergic neurons. *Neurosci* 1993; 56: 53–60.

130. Boydenkamp KE, Hoffman AE, Gerhardt HA, Henry MA, Biddle PT, Hoffman BJ, et al. Glial cell line-derived neurotrophic factor supports survival of injured midbrain dopaminergic neurons. *J Comp Neurol* 1995; 355: 479–489.

131. Banker GA. Trophic interactions between astroglial cells and hippocampal neurons in culture. *Science* 1980; 209: 809–810.

132. Noble M, Fok-Seang J, Cohen J. Glia are a unique substrate for the in vitro growth of central nervous system neurons. *J Neurosci* 1984; 4: 1692–1699.

133. Schmeckebach C, Miller HW. Astroglia-neuron interactions that promote long-term neuronal survival. *J Chem Neuroanat* 1993; 6: 229–237.

134. Hatten ME, Mason CA. Neuron-astroglial interactions in vitro and in vivo. *Trends Neurosci* 1986; 168–174.

135. Miller C, Tsao T, David S. Dibutyryl CMP, interleukin-1, and macrophage conditioned medium enhance the ability of astrocytes to promote neurite growth. *J Neurosci Res* 1994; 38: 56–63.

136. Dugan LE, Brosnan SM, Vaca K, Cuenod M, Mallat M. Glia modulate the response of murine cortical neurons to excitotoxicity: role of nerve growth factor. *J Neurochem* 1994; 61: 153–162.

137. Kimelberg HK, Norenberg MD. Microglia. In: *Handbook of Clinical Neurology*. Amsterdam: North-Holland; 1976: 401–421.

138. Nagata K, Tikei N, Nakajima K, Saito H, Kohsaka S. Microglial conditioned medium promotes survival and development of cultured mesencephalic neurons from embryonic rat brain. *J Neurosci Res* 1993; 34: 357–363.

139. Marlow M, Houlgatte R, Bruchet P, Frochoniat A. Lipopolysaccharide-stimulated brain macrophages release NGF in vitro. *Dev Biol* 1989; 133: 309–311.

140. Chamburin D, Mallat M. Immunohistochemical detection of threobromopin in microglia in the developing rat brain. *Neuroscience* 1995; 69: 177–187.

141. Chamburin D, Morandi V, Mallat M. Brain macrophages stimulate neurite growth and regeneration by secreting threobromopin. *J Neurosci Res* 1994; 38: 221–233.

142. Shimono M, Chiyojima K, Takii N, Hamanoue M, Kohsaka S. Production of basic fibroblast growth factor in cultured rat brain microglia. *Neurosci Lett* 1991; 123: 229–231.

143. Arai T, Yamada H, Endo Y, Kimura M, Kato Y. Fibroblast growth factor stimulates the differentiation of dopamine neurons. *J Neurocytol* 1993; 22: 13–16.

144. Gage FH. Cytokine regulation of nerve growth factor-mediated cholinergic neurotrophic activity synthesized by astrocytes and fibroblasts. *J Neurosci* 1992; 59: 919–931.

145. Gage FH. Cooperative regulation of nerve growth factor synthesis and secretion of fibroblasts and astrocytes by fibroblast growth factor and other cytokines. *Brain Res* 1992; 569: 14–25.

146. Merill JE. Tumor necrosis factor alpha, interleukin 1 and related cytokines in brain development: normal and pathological. *Dev Neurol* 1992; 14: 1–10.

147. Strijbos PJ, Anderson JD, Southwell NJ. Interleukin-1B attenuates excitatory amino acid-induced neurodegeneration in vitro: involvement of nerve growth factor. *J Neurosci* 1995; 15: 3468–3474.

148. Vaca K, Wende D. Divergent effects of astroglial and microglial secretions on neuron growth and survival. *Exp Neurol* 1991; 119: 346–351.

149. Akaneya T, Tatsumi M, Hatanaka H. Interleukin-1B enhances survival and interleukin-6 protects against MPTP neurotoxicity in cultures of fetal rat dorsal root ganglion neurons. *Exp Neurol* 1995; 136: 44–52.

150. Giulian D. Corpuz M. Microglial secretion products and their impact on the nervous system. *Adv Neurol* 1993; 59: 315–320.

151. Giulian D, Vaca K, Noonan CA. Secretion of neurotoxins by mono-nuclear macrophages and astrocytes. *J Neurosci Res* 1993; 251: 1593–1596.

152. Awell K. The distribution of microglia and cell death in the fetal rat forebrain. *Dev Brain Res* 1991; 58: 1–12.

153. Hame DM, Perry VH, Gordon S. Immunohistochemical localization of a macrophage-specific antigen in developing mouse retina: phagocytosis of dying neurons and differentiation of microglial cells to form a regular array in the plexiform layers. *J Cell Biol* 1983; 97: 253–257.

154. Colton CA, Gilbert DL. Microglia, an in vivo source of reactive oxygen species in the brain. *Adv Neurol* 1993; 59: 321–326.

155. Van Muissink FL, Veenhuis R, Eikenboom P. Amyloid β protein primes cultured rat microglial cells for an enhanced-phorbol 12-myristate 13-acetate-induced respiratory burst activity. *J Neurochem* 1996; 66: 2468–2476.

156. Banati RB, Hoppe K, Gottmann K, Kreutzberg GW. Cytotoxicity of microglia. *GLIA* 1993; 80: 111–118.

157. Banati RB, Rothe G, Valet G, Kreutzberg GW. Detection of lysosomal cytotoxic proteases in microglia: flow cytometric measurement and histochemical localization of cathepsin B and L. *GLIA* 1993; 7: 451–458.

158. Colton AC, Gilbert DL. Production of superoxide by a CNS macrophage, the microglia. *FEBS Lett* 1987; 223: 284–288.

159. Therby C, Chamburin D, Mallat M. Free radical killing of neurons. *Eur J Neurosci* 1991; 3: 1155–1164.

160. Dickson DW, Maruni T, Yamamoto H. Immunohistochemical detection of NGF receptor mediated neurotrophic activity synthesized by astrocytes and fibroblasts in cultured rat brain microglia. *J Neurocytol* 1993; 22: 172–179.

161. Piani D, Frei K, Quang Do K, Cuenod M, Fontana A. Brain macrophages induce NMDA receptor mediated neurotoxicity in vitro by secreting NGF in the adult brain. *J Neurosci Res* 1993; 38: 162–169.

162. Vincenzi AM, Louw CK, Verheijen HJ, Thijlers FJH, Van DAM AR. Role of astrocyte-derived tissue plasminogen activator in the regulation of endothon stimulated nitric oxide production by microglial cells. *GLIA* 1998; 22: 130–137.
179. Gower DB. Modifiers of steroid-hormone metabolism: a review of their chemistry, biochemistry and clinical applications. J Steroid Biochem 1974, 5: 501–542.

180. Gower DB, Gower ME. Molecular mechanisms of steroid-hormone actions in the brain. Ann NY Acad Sci USA 1994; 738: 76–85.

181. Huang Z, Huang PL, Panahian N, Dilkara T, Fishman MC, Moskowitz MA. Effects of cerebral ischemia in mice deficient in neuronal nitric oxide synthase. J Cereb Blood Flow Metab 1994; 14: 1883–1885.

182. Dawson VL, Dawson TM. Nitric oxide neurotoxicity. J Chem Neuroanat 1996; 10: 179–190.

183. Burz EA, Hostager BS, Southern PJ. Macrophages in mice acutely infected with lymphocytic choriomeningitis virus are primed for nitric oxide synthesis. Microbial Pathogen 1994; 16: 283–295.

184. Campbell IL, Sami A, Chang CS. Expression of inducible nitric oxide synthase. Correlation with neurology and clinical features in mice with lymphocytic choriomeningitis. J Immunol 1994; 153: 3622–3629.

185. Van Dam L, Dauer J, Buchkremer K, Marquenie C, Tilders EJH, Berkenbosch F. Appearance of inducible nitric oxide synthase in the rat central nervous system after rabies virus infection and during experimental allergic encephalomyelitis but not after peripheral administration of endotoxin. J Neurosci Res 1995; 40: 251–260.

186. Schwenk HM. Effect of chronic nitric oxide synthase blockade on local hypothalamic blood flow in rats. Brain Res 1995; 697: 3622–3629.

187. brainstorm E. Nitric oxide: first in a new class of neurotransmitters? Science 1993; 259: 465–467.

188. Schuman EM, Madison DV. A requirement for the intercellular messenger nitric oxide in long-term potentiation. Science 1993; 258: 1503–1506.

189. Ohno M, Yamamoto T, Watanabe S. Deficits in working memory following inhibition of hippocampal nitric oxide synthase in the rat. Brain Res 1994; 65: 111–115.

190. Bredt DS, Glatt CE, Hwang PM, Fotuhi M, Dawson TM, Snyder SH. Nitric oxide synthase protein and mRNA are discretely localized in neuronal populations of the mammalian CNS together with NADPH diaphorase. Neuron 1992; 14: 165–167.

191. Hawkins RD. NO honey, I don't remember. Neuron 1996; 16: 465–467.

192. Benyo Z, Szabo C, Stuiver BT, Bohus B, Sandor P. Effect of chronic nitric oxide synthase blockade on local hypothalamic blood flow in rats. Brain Res 1995; 697: 3622–3629.

193. Schuman EM, Madison DV. A requirement for the intercellular messenger nitric oxide in long-term potentiation. Science 1993; 258: 1503–1506.

194. Ohno M, Yamamoto T, Watanabe S. Deficits in working memory following inhibition of hippocampal nitric oxide synthase in the rat. Brain Res 1994; 65: 111–115.
Nitrergic synapses may be involved in the control of neuronal plasticity and synaptic transmission in the brain. Neuron 2001; 31: 3–9.

281. Plata-Salaman CR, Huganir RL. The involvement of the nNOS and eNOS enzymes in neurotransmitter and neuromodulator release in the central nervous system.

282. Schwartz JP, Wilson DJ. Preparation and characterization of type 1 astrocytes cultured from adult rat cortex, cerebellum and striatum.

283. Frei K, Lins H, Schwerdel C, Fontana A. Antigen presentation in the central nervous system: the inhibitory effect of IL-10 on MHC Class II expression and production of cytokines depends on the inducing signals and the type of cell analyzed.

284. Giulian D, Baker TJ. Characterization of ameboid microglia isolated from developing mammalian brain.

285. Wesselhöft SL, Takahashi K, Glass KD, McArthur JC, Griffin JW, Griffin DE. Cellular localization of tumor necrosis factor receptor mRNA in neurological tissue without detectable TNF-α.

286. Yoshida M, Bradley WG, Shapshak P, Nogawa I, Stewart BV, Xin, K. et al. The role of immune activation and cytokine expression in human immunodeficiency virus-related brain disease: a review. J Neuroimmunol 1995; 53: 355–358.

287. Perrella O, Finelli L, Carri MB. The role of cytokines in AIDS dementia. Curr Opin Neurol 2003; 16: 342–344.

288. Sippel BD, Hofman PM, Wallach D, Hofman FM. Increased expression of tumor necrosis factor-alpha receptors in the brains of patients with AIDS. J AIDS Hum Retrov 1995; 10: 511–512.

289. Bukrinsky MI, Nutt EHL, Schmidtmeyerova H, Dubovytsk V, Flejner I. Regulation of nitric oxide synthase activity in human immunodeficiency virus type 1 (HIV-1) infected monocytes: implications for HIV-associated neurological disease. J Exp Med 1995; 181: 735–745.

290. Colasanti M, Persichini T, Di Pucchio T, Gremo E, Lauro GM. Human ramified microglial cells produce nitric oxide upon E. coli lipopolysaccharide and tumor necrosis factor alpha stimulation. Neu- rosci Lett 1995; 200: 144–146.

291. Dhopeta R, Reu X, Huw JF, Filler AM, Courouy G, Gourea O. Expression of inducible nitric oxide synthase activity in cytomegalovirus-infected glial cells of retina from AIDS patients. Neurosci Lett 1995; 166: 31–34.

292. Huron S, Boissiere E, Faucheux B, Brugg B, Magniot-Paigne A, Agid Y, et al. Nitric oxide synthase and neuronal vulnerability in Parkinson’s disease. Neuroscience 1996; 72: 355–363.

293. Connop BE, Boergman RJ, Beninger RJ, Jamandals A. Attenuation of malneutro-derived induction of the neural growth by inhibi- tor of nitric oxide synthase. Neuropharmacology 1996; 35: 459–465.

294. Ichichoupol D, Huran D, Horwitz J. Peroxynitrite-mediated inhibition of DOPA synthesis in PC12 cells. J Neurochem 1995; 65: 2366–2372.

295. Paakkan L, Lindberg P. Nitric oxide in the central nervous system. Ann N Y Acad Sci 1995; 769: 369–377.

296. Endoh M, Muies K, Wagner J. Induction of the expression of nitric oxide synthase by reactive astrocytes after transient global ischemia. Brain Res 1997; 651: 92–100.

297. Nowicki JP, Deval D, Puigser S, Scotton B. Nitric oxide mediates neurocellular communication after focal cerebral ischemia in the mouse. Eur J Pharmacol 1991; 204: 339–340.

298. Berini G, Savio T, Zache D, Schmidt HB, Bentoviglio M. NADPH diaphorase activity in brain microvascular pericytes after streptozotocin-induced diabetes in the rat. Neurosci Lett 1993; 152: 95–98.

299. Wallace MN, Fredens K. Activated astrocytes of the mouse hippo- campus contain high levels of NADHdiaphorase. Neuroreport 1992; 3: 953–956.

300. Glenn J, Adan FL, Thomas WE. Further studies on the identification of microglia in mixed brain cell cultures. Brain Res Bull 1989; 22: 1049–1052.

301. Hassan NE, Campbell DE, Rifat S, Douglas SD. Isolation and character- ization of human fetal brain-derived microglia in vitro. J Neuroimmunol 1991; 31: 153–159.

302. Gebicki-Haerter PJ, Bauer J, Schobert A, Nordhoff H. Lipopolysaccha- ride-free conditions in primary astrocyte cultures allow growth and isolation of microglial cells. J Neurosci 1989; 9: 183–194.

303. Thiele DE, Malekova M, Molkentin JD. Neurotensin is a high affinity cell culture for mononuclear-stimulated T and B cell responses in human peripheral blood: delineation by its sensitivity to the lymphosorotopic agent, Léucine methyl ester. J Immunol 1985; 131: 2282–2290.

304. McCarrey JD, Velle D. Preparation of separate astroglial and oligodendroglial cell cultures from rat cerebral tissue. J Cell Biol 1980; 85: 390–92.

305. Schwartz JP, Wilson DJ. Preparation and characterization of type 1 astrocytes cultured from adult rat cortex, cerebellum and striatum. GLIA 1992; 5: 75–80.

306. Frei K, Lins H, Schwedel C, Fontana A. Antigen presentation in the central nervous system: the inhibitory effect of IL-10 on MHC Class II expression and production of cytokines depends on the inducing signals and the type of cell analyzed. J Immunol 1994; 152: 2720–2728.

307. Giulian D, Baker TJ. Characterization of ameboid microglia isolated from developing mammalian brain. J Neurosci 1986; 6: 2163–2178.

308. Suzuki M, Mizutani S, Gonatas NK, Silverberg DH, Kricheff MI. Expression of tumor necrosis factor-alpha mRNA by microglia from newborn mouse brain: induction of tumor necrosis factor-alpha by gamma-mercaptoethanol. J Neuroimmunol 1987; 15: 265–278.

309. Jordan FE, Verbeke WE. Identification of microglia in primary cultures of mixed cerebral cortical cells. Brain Res Bull 1987; 19: 153–159.

310. Sutter A, Gerdes C, Larling F. Microglial contaminants as the source of IL-1 in astrocyte cultures. Abstract First European Meeting on Glial Cell Function in Health and Disease, Heidelberg, 1994; Match 24–27, 1994.

311. Demerie-Pallardy L, Loncmand P, Chabrier P. Nitric oxide synthase induction in glial cells: effect on neuronal survival. Life Sci 1993; 52: 1883–1890.

312. Zielasek J, Tausch M, Toyka KV, Hartung H. Production of nitrite by
Galea E, Reis DJ, Fox ES, Xu H, Feinstein DL. CD14 mediates endotoxin induction of nitric oxide synthase in cultured brain glial cells. *Neurochem Res* 1992; 17: 229–232.

Galea E, Reis DJ, Fox ES, Xu H, Feinstein DL. CD14 mediates endotoxin induction of nitric oxide synthase in cultured brain glial cells. *Neurochem Res* 1992; 17: 229–232.

Corradin SB, Mauel J, Donini SD, Quattrocchi E, Ricciardi-Castagnoli P. Inducible nitric oxide synthase activity of cloned murine microglial cells. *J Neurochem* 1996; 66: 1419–1423.

Perretti M, Szabo C, Thiermermann C. Effect of interleukin-4 and interleukin-10 on leucocyte migration and nitric oxide production in the mouse. *Br J Pharmacol* 1995; 116: 2251–2257.

Liew FY, Li Y, Severn PJ, Salter M, et al. A possible novel pathway of regulation by murine T helper type-2 (Th2) cells of a Th1 cell activity via the modulation of the induction of nitric oxide synthase on macrophages. *J Immunol* 1991; 147: 2489–2494.

Young MRI, Farietta T, Crayton JW. Production of nitric oxide and transforming growth factor-β in a mediator of central nervous system dysfunction in acquired immune deficiency syndrome. *J Exp Med* 1993; 173: 981–991.

Park SK, Lin HL, Murphy S. Nitric oxide limits transcriptional induction of nitric oxide synthase in brain macrophages. *J Neurochem* 1995; 64: 194–201.

Hu S, Sheng WS, Peterson PK, Chao CC. Differential regulation by cytokines of production of nitric oxide by human astrocytes. *J Immunol* 1992; 149: 1473–1481.

Lipton SA. Requirement for macrophages in neuronal injury induced by HIV envelope protein gp120. *Brain Pathol* 1995; 5: 291–300.

Kuruvilla AP, Shah R, Hochwald GM, Liggitt HD, Palladino MA, Thorbecke GJ. Protective effect of transforming growth factor-β isotypes in multiple sclerosis: differential glial expression of TGF-β1, 2 and 3 isotypes in multiple sclerosis. *J Neuroimmunol* 1996; 71: 115–123.

Flanders KC, Lippa CE, Smith TW, Pollen DA, Sporn MB. Altered expression of transforming growth factor-β in Alzheimer's disease. *Neurology* 1997; 45: 1561–1568.

Perretti NS, Perillo E. Differential expression of TGF-β1 and 3 isotypes in Alzheimer's disease: a comparative immunohistochemical study with cerebral inflammation, aged human and mouse control brains. *J Neuroimmunol* 1995; 54: 802–811.

Samuel-Van Asten JM, Beckman S, Panazza CG, Allen MB Jr. Immunocytochemical study of transforming growth factor expression in benign and malignant gliomas. *Am J Pathol* 1989; 134: 895–902.

Yamada N, Kato M, Yamashita H, Nister M, Miyazono K, Heldin C, et al. Enhanced expression of transforming growth factor-β and its type-I and type-II receptors in human glioblastoma. *Int J Cancer* 1995; 62: 386–392.

Chao CC, Hu S, Sheng WS, Peterson PK. Tumor necrosis factor-alpha production by human fetal macroglial cells: regulation by other cytokines. *J Neurochem* 1995; 64: 194–201.

Merrill JE, Zimmerman RP. Natural and induced cytotoxicity of oligodendrocytes by microglia is inhibitable by TGF-β. *GLIA* 1991; 4: 327–331.

Gilbert RS, Herschorn HR. Transforming growth factor beta differentially modulates the inducible nitric oxide synthase gene in distinct cell types. *Biochem Biophys Res Commun* 1993; 195: 380–384.

Ding A, Nathan CB, Graycar J, Derynick R, Stuehr DJ, Sirmal S. Macrophage nitric oxide synthase and activating factors of transforming growth factor-β, β2, β3 inhibit induction of macrophage nitrogen oxide synthesis by IFN-gamma. *J Exp Med* 1990; 265: 2053–2058.

Chao CC, Hu S, Sheng WS, Peterson PK. Tumor necrosis factor-alpha production by human fetal macroglial cells: regulation by other cytokines. *J Neurochem* 1995; 64: 194–201.

Merrill JE, Zimmerman RP. Natural and induced cytotoxicity of oligodendrocytes by microglia is inhibitable by TGF-β. *GLIA* 1991; 4: 327–331.

Wahl SM, Hunt DA, Wakefield IA, McCartney-Francis N, Wahl LM, Roberts AB, et al. Transforming growth factor beta (TGF-β) induces monocyte chemotaxis and growth factor production. *Proc Natl Acad Sci USA* 1987; 84: 5788–5792.

Racke MK, Dich-Jalbut S, Caturelli B, Albert PS, Raine CS, McFarlin DE. Prevention and treatment of chronic relapsing experimental allergic encephalomyelitis by transforming growth factor-β. *J Exp Med* 1991; 163: 3012–3017.

Kuruvilla AP, Shah R, Hochwald GM, Liggitt HD, Palladino MA, Thorbecke GJ. Protective effect of transforming growth factor-β isotypes in multiple sclerosis: differential glial expression of TGF-β1, 2 and 3 isotypes in multiple sclerosis. *J Neuroimmunol* 1996; 71: 115–123.

Flanders KC, Lippa CE, Smith TW, Pollen DA, Sporn MB. Altered expression of transforming growth factor-β in Alzheimer's disease. *Neurology* 1997; 45: 1561–1568.

Perretti NS, Perillo E. Differential expression of TGF-β1 and 3 isotypes in Alzheimer's disease: a comparative immunohistochemical study with cerebral inflammation, aged human and mouse control brains. *J Neuroimmunol* 1995; 54: 802–811.

Samuel-Van Asten JM, Beckman S, Panazza CG, Allen MB Jr. Immunocytochemical study of transforming growth factor expression in benign and malignant gliomas. *Am J Pathol* 1989; 134: 895–902.

Yamada N, Kato M, Yamashita H, Nister M, Miyazono K, Heldin C, et al. Enhanced expression of transforming growth factor-β and its type-I and type-II receptors in human glioblastoma. *Int J Cancer* 1995; 62: 386–392.

Chao CC, Hu S, Sheng WS, Peterson PK. Tumor necrosis factor-alpha production by human fetal macroglial cells: regulation by other cytokines. *J Neurochem* 1995; 64: 194–201.

Merrill JE, Zimmerman RP. Natural and induced cytotoxicity of oligodendrocytes by microglia is inhibitable by TGF-β. *GLIA* 1991; 4: 327–331.

Gilbert RS, Herschorn HR. Transforming growth factor beta differentially modulates the inducible nitric oxide synthase gene in distinct cell types. *Biochem Biophys Res Commun* 1993; 195: 380–384.

Ding A, Nathan CB, Graycar J, Derynick R, Stuehr DJ, Sirmal S. Macrophage nitric oxide synthase and activating factors of transforming growth factor-β, β2, β3 inhibit induction of macrophage nitrogen oxide synthesis by IFN-gamma. *J Exp Med* 1990; 265: 2053–2058.

Chao CC, Hu S, Sheng WS, Peterson PK. Tumor necrosis factor-alpha production by human fetal macroglial cells: regulation by other cytokines. *J Neurochem* 1995; 64: 194–201.

Merrill JE, Zimmerman RP. Natural and induced cytotoxicity of oligodendrocytes by microglia is inhibitable by TGF-β. *GLIA* 1991; 4: 327–331.

Wahl SM, Hunt DA, Wakefield IA, McCartney-Francis N, Wahl LM, Roberts AB, et al. Transforming growth factor beta (TGF-β) induces monocyte chemotaxis and growth factor production. *Proc Natl Acad Sci USA* 1987; 84: 5788–5792.

Racke MK, Dich-Jalbut S, Caturelli B, Albert PS, Raine CS, McFarlin DE. Prevention and treatment of chronic relapsing experimental allergic encephalomyelitis by transforming growth factor-β. *J Exp Med* 1991; 163: 3012–3017.

Kuruvilla AP, Shah R, Hochwald GM, Liggitt HD, Palladino MA, Thorbecke GJ. Protective effect of transforming growth factor-β on experimental autoimmune diseases in mice. *Proc Natl Acad Sci USA* 1991; 88: 2918–2921.

Karpus WJ, Sabo C, Swanson RH. CD4+ suppressor cells inhibit the function of effector cells of experimental autoimmune encephalomyelitis through a mechanism involving transforming growth factor-β. *J Immunol* 1994; 153: 1163–1168.

Johns LD, Sriram S. Experimental allergic encephalomyelitis: neutralizing antibody to TGF-β protects against allergic encephalomyelitis. *J Immunol* 1995; 151: 1163–1168.

Rumiano AL, Lekfieje S, Serrano A, Masson A, Benavides J, Zavala F. Biphasic transforming growth factor-β production flanking the pro-inflammatory cytokine response in cerebral response in cerebral trauma. *Neuro Report* 1995; 7: 133–136.

Mediators of Inflammation · Vol 7 · 1998

254
ISSAZADEH S, MUSTAFA M, LJUNGDAHL A, HOJEBERG B, DAGERLUND A, ELDE R, et al. Interferon gamma, interleukin 4 and transforming growth factor beta in experimental autoimmune encephalomyelitis in Lewis rats: dynamics of cellular mRNA expression in the central nervous system and lymphoid cells. J Neurosci Res 1995; 40: 579–590.

McNeill H, Williams C, Guan J, Dragmanow M, Lawlor P, Strimanne E, et al. Neuronal rescue with transforming growth factor-beta 1 after hypoxic-ischaemic brain injury. Neuro Report 1994; 5: 901–904.

Lindholm D, Castren E, Keifer R, Zafra E, Toenen H. Transforming growth factor-beta 1 in the rat brain: increase after injury and inhibition of astrocyte proliferation. J Cell Biol 1991; 117: 395–400.

Saad B, Constam DB, Ortmann R, Moos M, Fontana A, Schachner M. Astrocyte-derived TGF-beta 2 and NGF differentially regulate neuronal recognition molecule expression by cultured astrocytes. J Cell Biol 1991; 115: 473–484.

Behzadian MA, Wang X, Jiang B, Caldwell RB. Angiostatic role of astrocytes: suppression of vascular endothelial cell growth by TGF-beta and other inhibitory factor(s). Glia 1995; 15: 480–490.

Chao CC, Hu S, Sheng WS, Tsang M, Peterson PK. Tumor necrosis factor-alpha mediated the release of bioactive transforming growth factor-beta 2 in murine microglial cell cultures. J Immunol Immunopathol 1995; 77: 358–365.

da Cunha A, Jefferson JA, Jackson RW, Vitkovic L. Glial cell-specific mechanism of TGF-beta in murine microglial cell cultures. J Immunol Immunopathol 1995; 77: 358–365.

Lyons RM, Gentry LE, Purchio AF, Moses HL. Mechanism of activation of latent recombinant transforming growth factor-beta 2 and NGF differentially regulate neuronal recognition molecule expression by cultured astrocytes. J Cell Biol 1994; 117: 395–400.

McNeill H, Williams C, Guan J, Dragmanow M, Lawlor P, Strimanne E, et al. Neuronal rescue with transforming growth factor-beta 1 after hypoxic-ischaemic brain injury. Neuro Report 1994; 5: 901–904.

Lindholm D, Castren E, Keifer R, Zafra E, Toenen H. Transforming growth factor-beta 1 in the rat brain: increase after injury and inhibition of astrocyte proliferation. J Cell Biol 1991; 117: 395–400.