It has been recognized for many years that immunological responses in the central nervous system (CNS) are different from those that occur at most other sites in the body [1]. Among the factors that are thought to contribute to this immunologically privileged status of the CNS are the specialized vasculature that restricts access of inflammatory cells and the lack of expression of MHC molecules by neurons and most glia [2,3]. However, once an immune response has been initiated in the CNS, it may proceed with devastating effects on neuronal and glial function. A key question in the analysis of these events is the extent to which resident glial cells mediate or regulate the inflammatory response. Glial cells of the CNS, especially microglia and astrocytes, are both the target and source of cytokines in CNS inflammation [4,5]. In addition, astrocytes are known to produce inflammatory mediators such as NO and arachidonic acid metabolites [6,7]. The present review focuses on the interaction between microglia and astrocytes through components of the interleukin (IL)-1 system to regulate astrocyte high-output NO production.

NO is a molecule with pleiotropic effects [8]. It is generated from L-arginine by nitric oxide synthases (NOS), of which there are three isoforms. The constitutive forms (NOS I and III) are primarily expressed in neurons and endothelial cells, and have been shown to mediate neurotransmission and vasodilation respectively. They are Ca²⁺- and calmodulin-dependent and produce low levels of NO in response to transient increases in intracellular Ca²⁺. NOS III or inducible NOS was first characterized in murine macrophages and subsequently shown to be expressed in a variety of cell types. Activation signals for NOS II are cell-type- and species-specific, but generally include immunological stimuli such as cytokines, bacterial products and immune complexes [9]. Activation of NOS II is transcriptionally regulated and...
Ca\textsuperscript{2+}-independent. Induction of NOS II results in sustained release of NO leading to much higher levels of production than that of the constitutive enzymes, resulting in toxicity for both pathogenic organisms and host cells. In addition to mediating cytotoxicity, NO has also been shown to be immunosuppressive through its anti-proliferative effect on lymphocytes [10], down-regulation of MHC class-II molecules [11] and inhibition of adhesion of inflammatory cells to vascular endothelium [12].

NO has been implicated in the pathogenesis of several CNS diseases such as Alzheimer's, multiple sclerosis [13,14] and HIV encephalitis [15]. In support of this, in vitro studies have shown significant toxicity of glial-cell-derived NO to both oligodendrocytes and neurons [16,17]. However, studies in the animal model of multiple sclerosis, experimental autoimmune encephalomyelitis (EAE), have resulted in conflicting conclusions about the role of NO in the pathogenesis of disease. The treatment of the SJL mouse with EAE by Cross et al. [18] using a NOS inhibitor, aminoguanidine, halted disease progression in the chronic phase of EAE, suggesting a pathogenic role for NO. However, in a study of the NOS II knock-out mice sensitized to develop EAE, increased disease severity compared with wild-type animals was observed, and these mice did not recover from the acute phase of the disease [18a], indicating a protective or immunosuppressive role for NO. The differences in these studies probably reflect a biphasic role for NO in the course of disease, with NO contribution to both initiating and regulatory events. Thus it is likely that the ultimate effect of NO on the course of disease is determined by a multitude of factors including the kinetics and levels of production.

One of the central issues of NO biology is the extent to which the data in rodent systems pertain to human disease, because NOS appears to be regulated in a cell-type- and species-specific manner. In the rodent CNS, both microglia and astrocytes have been shown to express type-II NOS, with microglia providing a greater source of NO [19]. In contrast, our own studies of primary human glia showed that microglia do not express NOS II in response to a variety of stimuli, including combinations of cytokines and lipopolysaccharide (LPS) [6]. Human astrocytes, in contrast, are readily induced by cytokines to express NOS II and produce high levels of NO similar to rodent macrophages and microglia. In both rodent and human cells it has been shown that a combination of cytokines and/or bacterial products is the most effective activating signal for NOS II expression. It is thought that the requirement for more than one signal provides an additional protective mechanism for the host from the detrimental effects of this highly reactive radical. It is noteworthy that in contrast with rodent astrocytes and microglia, in which a combination of LPS and cytokines results in maximal activation of NOS II, human astrocytes do not respond to LPS [5,6]. No induction of NOS mRNA or enzyme activity was found in human fetal astrocytes when exposed to LPS alone or in combination with cytokines. Another difference between rodent macrophages and human astrocytes is the requirement for IL-\textbeta for astrocyte NOS II induction. A similar pivotal role for IL-1\textbeta has also been discovered for NOS II induction in human hepatocytes [20]. In human astrocyte cultures, we observed that, whereas IL-1\textbeta alone stimulated a low level of NO production and interferon \gamma (IFN-\gamma) alone had no effect, there was a striking synergistic response when the two were used in combination. Astrocytes treated with IL-1\textbeta + IFN-\gamma produced levels of NO similar to that found in rodent microglia. Tumour necrosis factor \alpha (TNF-\alpha) and IL-6, either alone or in combination with IFN-\gamma, failed to induce NO. However, IL-1\textbeta + TNF-\alpha showed a moderate increase in NO production over levels with IL-1\textbeta treatment alone [6,21]. The combination TNF-\alpha + IL-1\textbeta + IFN-\gamma induced maximal levels of NO. Thus IL-1\textbeta appears to be critical for induction of NOS II expression in human astrocytes. In addition to inducing the production of NO, the combination of IL-1\textbeta + IFN-\gamma also activates TNF-\alpha production by human astrocytes [5], another potentially cytotoxic cytokine that has been implicated in both multiple sclerosis and HIV-1 encephalitis.

It is interesting that in human astrocytes, the kinetics of NOS II expression and NO production were somewhat different from those found in other cell types. In cultures of human astrocytes, NO was barely detectable before 24 h of cytokine stimulation, but the levels then steadily rose for as long as 7 days in culture [21]. Similarly, the kinetics of NOS II mRNA expression showed that, once stimulated, the mRNA accumulated for 48 h after stimulation and showed high sustained levels at 72 h, the longest time point examined. This is in contrast with the kinetics of NO production in other human cell
types, as well as in rodent astrocytes and microglia, which shows a decrease 72 h after stimulation, which is probably due to an NO-dependent negative feedback mechanism and/or depletion of the substrate L-arginine in the culture medium [22-25].

Because of the lack of intrinsic down-regulation of NO production in human astrocytes stimulated with IL-1β and IFN-γ, we sought to determine whether other cytokines given exogenously might inhibit expression of NOS II in astrocytes. We examined the effect of pretreatment of human astrocytes with TGF-β, IL-4 or IL-10, all cytokines known to inhibit NOS II induction in rodent macrophages and glia. The results showed that none of them had any effect on NOS II expression in human astrocyte cultures [6]. However, the same cells up-regulated monocyte chemoattractant peptide-1 after treatment with these ‘inhibitory’ cytokines, eliminating the possibility that the lack of inhibition of NO production was due to lack of receptors or response to these cytokines [6].

The only cytokine effective in inhibiting the IL-1-mediated activation of NO production was found to be IL-1 receptor antagonist (IL-1Ra). IL-1Ra is a naturally occurring inhibitor of IL-1 [26, 27]. It competes with IL-1 for IL-1 receptors, but its binding does not result in signal transduction. We have observed the complete abrogation of the induction of NOS II transcription and NO production when IL-1Ra is used at a 200-fold molar excess to IL-1β, supporting the critical role of IL-1 in the activation of NOS II in astrocytes [6].

Thus it appears that regulation of NO production in human astrocytes is determined by the balance of agonists and antagonists of the IL-1 system. The components of the IL-1 system consist of three members of the IL-1 family, IL-1β-converting enzyme (ICE) and the type-I and type-II receptors [27]. There are three known members of the IL-1 family, which share similar structural topology despite considerable differences in sequence. IL-1α and IL-1β bind to the same receptors and in most systems show the same biological activity. Both are primarily the products of cells of the monocyte/macrophage lineage. IL-1α does not require proteolytic cleavage for activation and remains mostly cytosolic. However, it also has a membrane-bound form which is biologically active. IL-1β, on the other hand, is produced as inactive pro-IL-1β and requires cleavage by ICE for secretion and activity. It is IL-1β rather than IL-1α that is implicated in systemic conditions such as sepsis, since only the IL-1β is detected in the circulation [27]. The final member of the IL-1 family is IL-1Ra, which is a naturally occurring antagonist of IL-1 action. In the periphery, both monocyte-macrophages and neutrophils are the major source of IL-1Ra. IL-1Ra was first described to be induced in monocytes exposed to immobilized immunoglobulins, and was subsequently shown to be induced by LPS and cytokines as well.

In peripheral blood monocytes and macrophages, various inflammatory mediators have been shown to differentially regulate IL-1 and IL-1Ra [28-30]. LPS induces both IL-1 and IL-1Ra. IL-1 itself also induces both IL-1 and IL-1Ra. Haemopoietic cytokines including IL-3 and granulocyte/macrophage colony-stimulating factor are potent inducers of IL-1Ra, but do not affect IL-1 production. IL-4, used alone, induces the production of IL-1Ra but not IL-1β.

Since microglia are derived from the monocyte/macrophage lineage, they are a potential source of both IL-1β and IL-1Ra in the CNS and may be pivotal in the induction and suppression of astrocyte activation for NOS and TNF-α expression. Our study of primary human microglia has shown that various cytokines, LPS and HIV-1/gp120 differentially regulate the production of IL-1β and IL-1Ra ([5]; C. F. Brosnan, S. C. Lee and J. Liu, unpublished work). When stimulated with LPS, microglia express abundant mRNA and protein for both IL-1β and IL-1Ra. A comparison of the effect of exposure to HIV and the LPS response showed that the kinetics and magnitude of IL-1β induction by these two stimuli were similar. However, unlike LPS stimulation, HIV (gp120) did not induce IL-1Ra production. Since productive HIV infection occurs several days after exposure of microglia to the virus [31], the rapid kinetics (8-26 h) of IL-1β induction may be the result of surface interaction between the virus and a microglial cell-surface receptor. gp120, the HIV envelope glycoprotein known to interact with CD4 and perhaps the chemokine receptor, was found to stimulate IL-1β production in microglia with the same kinetics as the whole virus.

As shown for the LPS-stimulated monocyte and macrophages in the periphery, IL-1β produced by microglia in response to LPS or HIV-1/gp120 remains primarily cell-associated and is not released into the culture medium ([5]; C. F. Brosnan, S. C. Lee and J. Liu, unpublished
work). The lack of secretion suggests that ICE is not activated in microglia by the same signals that induce IL-1β. ICE is present constitutively as a proenzyme in cells of the monocyte/macrophage lineage and must be processed for activation [27]. The signals and mechanisms for ICE activation have not been defined. Clearly, identification of such factors would be of critical importance in understanding the biology of IL-1 in the CNS [32].

In human microglial culture, IL-1Ra is constitutively produced and secreted into the culture medium. IL-4, used alone, up-regulated the expression of IL-1Ra by human microglia but had no effect on IL-1β production. When given in combination with LPS, IL-4 dramatically increased the production of IL-1Ra and down-regulated LPS-induced IL-1β. In contrast, neither TGF-β nor IL-10 induced IL-1Ra production, but IL-10 exerted a down-regulatory effect on LPS-stimulated IL-1β production whereas TGF-β had no effect. Thus IL-4 appears to be unique, acting as an antagonist to IL-1β both by inhibiting IL-1β production and by stimulating the production of IL-1Ra.

The two IL-1 receptors may also function to modulate the effect of IL-1 in the CNS. All members of the IL-1 family bind to the type-1 IL-1 receptor with equal affinity. IL-1 receptor type I is capable of transducing signal when bound by either IL-1α or IL-1β. IL-1 receptor type II is structurally similar to type I except that its cytoplasmic domain is truncated and probably does not transduce signal on binding. It has been suggested that the type-II receptor is a decoy that functions to absorb IL-1, preventing interaction with the type-I receptor. The IL-1 receptors have not yet been studied in human glia, but it is likely that their expression could further influence the IL-1 response.

In inflammatory CNS conditions, it is likely that the nature of the infiltrating helper T-cell (Th1 compared with Th2) is critical for the regulation of the IL-1 effect and NO production. A Th1 helper response induces the production of IFN-γ, a cytokine necessary for the efficient activation of NO and TNF-α production in astrocytes. Th1 helper cells also activate peripheral blood macrophages that infiltrate the CNS and thus function as an additional source of IL-1β and TNF-α. In contrast, IL-4, the predominant cytokine produced by Th2 helper T-cells, may shift the balance toward antagonism of IL-1 by augmenting the synthesis of IL-1Ra and inhibit-
The immune response in the Alzheimer's disease brain

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'Alzheimer's disease' – a valid diagnostic entity?
The rapid change in diagnostic concepts in psychiatry has resulted in a succession of different disease classifications (ICD9, ICD10, DSM-III, DSM-III-R, DSM-IV [1]), which are based on expert consensus and operationalized diagnostics, with a tendency to increasingly replace the concept of 'disease' by the concept of 'disorder', implying a more heuristic approach. In the case of Alzheimer's disease (AD), the American Psychiatric Association published clinical criteria in DSM-III (1980), revised in DSM-III-R and again in DSM-IV (1987) [1], demanding that for the diagnosis of AD, the patient's memory and some other cognitive and/or personality deficit (in the absence of any disturbance of consciousness) has to become sufficiently severe to interfere with social life or work. One can easily perceive how a diagnosis on this basis is subject to imponderables such as variation in education and premorbid cognitive capacity. Hence much emphasis is placed on the neuropathological assessment of patients with dementing disease. This assessment is based on the densities of senile plaques and neurofibrillary tangles in neocortical areas [2]. However, even these criteria are subject to the initial clinical diagnosis, since they are recommended to be revised by 50% in the presence of a positive clinical history of AD. In addition, a large proportion of the patients have another neuropathological diagnosis as well [3]. In the absence of specific clinical markers and the many unknowns concerning the frequency of certain genotypes associated with an increased risk of AD [4], one proposed strategy, particularly in the assessment of the efficiency of anti-dementia drugs, is the definition of clinical subgroups, e.g. by multivariate statistical analysis of the treatment response. Such an approach supported by neuroimaging techniques, as recently suggested by the Organizing Committee of the Canadian Consensus Conference on the Assessment of Dementia [5], would effectively revert the all-inclusiveness of the currently used diagnostic criteria of AD. This is not without historical precedence since the contemporary debate that surrounded the first description of senile plaques and neurofibrillary tangles in human autopsy material focused on the question of whether these findings on their own are enough to establish a disease entity or whether an early onset of the disease (and with it the characteristic neuropathological phenomenology) was mandatory to form a meaningful classification different from so-called normal aging or conditions such as cerebrovascular disease, the standard diagnosis