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The Multifaceted Profile of Activated Microglia

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Abstract Although relatively neglected previously, research efforts in the past decade or so have identified a pivotal role for glial cells in regulating neuronal function. Particular emphasis has been placed on increasing our understanding of the function of microglia because a change from the ramified "resting" state of these cells has been associated with the pathogenesis of several neurodegenerative diseases, notably Alzheimer's disease. However, it is not clear whether activation of microglia and the associated inflammatory changes play a part in triggering disease processes or whether cell activation is a response to the early changes associated with the disease. In either case, the possibility exists that modulation of microglial activation may be beneficial in some circumstances, underlying the need to pursue research in this area. The original morphological categorization of microglia by Del Rio Hortega into ameboid, ramified, and intermediate forms, must now be elaborated to encompass a functional description. The evidence which has been generated recently suggests that microglia are probably never in a "resting" state and that several intermediate transitional states, based on function and morphology, probably exist. A more complete understanding of these states and the triggers which lead to a change from one to another state, and the factors which modulate the molecular switch that determines the persistence of the "activated" state remain to be identified.

Keywords Microglia · Cytokines · Cell surface markers · Phagocytosis · Cell–cell interactions

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Background

Glia, which include astrocytes, oligodendrocytes, ependymal cells and microglia, far outnumber neurons in the central nervous system (CNS) but interest in understanding their functions remained largely unexplored until relatively recently. In the past 15 or so years, however, it has become abundantly clear that the original role of glia, that is to provide support for neurons, is a profound oversimplification and an inaccurate description of their function. This paper reviews some of the changes which occur when microglia morph from their ramified state, which is usually described as their "resting" state, and discusses the data which indicate that microglia are likely to exist in a number of different states.

Microglia comprise 10–20% of all cells in the nervous system, but they are less numerous in white matter than in gray matter. Microglia are modified in both their morphology and surface antigen expression by their microenvironment [1–3]. They are the major resident immunocompetent cells in the brain [4] and are thought to derive from early embryonic macrophage-like mesodermal precursor cells. Therefore, they share the phenotypic markers of monocytes and macrophages, many of which are surface antigens with significant functional properties [5]. It is widely recognized that a change in microglial activation state occurs in experimentally-induced stress and in animal models of neurodegenerative disease [6]. Under these conditions, microglial cells react by releasing immunomodulatory molecules which can contribute to neuronal dysfunction and cell death [7]; these released molecules include inflammatory proteins like cytokines and chemokines, reactive oxygen and nitrogen species, and complement factors. The overwhelming evidence is that microglia coordinate the inflammatory response in the CNS and, by

means of expressing cell surface markers and releasing chemokines, orchestrate the infiltration of peripheral immune cells as part of the inflammatory response [8].

Microglia at Rest and Following Activation

It is probably a misnomer to apply the term "resting" to microglia since it seems that they are continually involved in surveillance and microglial processes, which are largely responsible for this function, are therefore continually motile. Under these conditions, the cells (apart from the processes) are not motile and there is minimal expression of cell surface markers and release of cytokines and chemokines, and the cells are not involved in phagocytosis. However, as they clearly are not at rest, microglia under these circumstances might be better described as *monitoring* microglia.

It is probably accurate to state that the function of resting microglia is largely unknown although key roles in homeostasis, host defense and repair have been attributed to these cells [9]. It has been hypothesized that microglia are responsible for dealing with the microdamage that occurs commonly in the brain and that this ranges from forms of plasticity which are associated with resculpting (perhaps eliminating) synapses to damage to capillaries [10]. As the major resident immunocompetent cells in the brain [4, 11], they are responsible for sampling the microenvironment and play a role in removing cell debris [9]. This surveying role is likely to be an important factor in maintenance of homeostasis; microglia possess an array of ion channels, and expression and activation potassium channels, in particular, have been suggested to be important in production of inflammatory mediators in response to stressors [12] and neuronal survival/death [13]. Similarly, at least in certain circumstances, microglia, like astrocytes, express the glutamate uptake protein, GLT-1, suggesting that they may play a role in protection against glutamate toxicity [14]. Ex vivo studies have also identified a key role for microglia in host defense against *Toxoplasma gondii* [15] and also against parasitic infections [16]. It is important to emphasize that these functions of microglia may require some degree of activation and therefore the issue of the functions of resting cells remains elusive. Similarly, the described role for microglia in repair has been coupled with their ability to produce neurotrophic factors, although it is also unclear whether release of neurotrophins can be achieved by "resting" cells [17]. What is clear is that microglia are exquisitely sensitive to stressors and rapidly change their morphology and function in response to all forms of insult.

There is lack clarity in the literature on how activated microglia might be accurately described. Del Rio Hortega identified the fact that significant morphological changes occurred when these cells were activated and suggested that microglial cells could be divided into three types; ameboid, ramified, and intermediate forms. However, the likelihood is that several functionally distinct activation states exist, rather than the limited number described by Del Rio Hortega based on morphology. Microglia can be stimulated and may then express particular cell surface markers which promote chemotaxis or infiltration of circulating cells into the CNS which permit interaction of microglia with other cells. They may be alternately non-phagocytic cells producing soluble proinflammatory molecules or they may be phagocytic and motile [18–20]. Intermediate states may also exist; for example, it has been proposed that expression of major histocompatibility complexes (MHC) enables phagocytic function and that engulfment of pathogens or cell debris promotes release of soluble proinflammatory molecules [21] (Table 1). Although no similar study has been undertaken in microglia, it is interesting to note that phagocytic macrophages which express Fc and scavenger receptors did not appear to be involved in antigen presentation but released certain chemokines and cytokines in a time-dependent manner [22]. These findings highlight the fact that characterization of the activation state of macrophages is complex; the similarities in the functions of microglia and macrophages suggest that a similar complexity in terms of activation states may be shared by microglia.

Cell Surface Markers Which Indicate an Altered Activation State of Microglia

Among the cell surface markers which are expressed on stimulated microglia are MHCI and MHCII, CD40, CD11b, F_c receptors I–III, complement receptors (CR1, 2, and 4), β_2 integrins, the costimulatory molecules CD80, CD86, and intercellular adhesion molecule-1 (ICAM-1) [23–27]. These markers have been variously used as indicators of microglial activation but the limitations of their use are seldom recognized. Important among these limitations is that several (if not all) of the markers are not unique to microglia and that, except in particular circumstances for example where pure microglial cultures are used, their upregulation may not reflect changes in microglia alone (Table 2). It is also important to recognize that, to a large extent, studies report changes in expression of cell surface markers under various conditions with minimal assessment of functional change and therefore the coupling of phenotype and function remains a challenge.

MHCII

Expression of MHCII antigens is the hallmark of antigen-presenting cells, and their expression on activated cells, when costimulatory molecules are also expressed, is

Table 1 At least five functional states of microglia exist but intermediate states, and states which are multifunctional, probably also exist

Description	Morphology/function
Resting (monitoring)	Extensive processes
	Sampling/surveying environment
Phagocytic	Ameboid structure with retracted processes, large cell body, and nucleus
	Motile
	CD68-expressing (indicative of lysosomal activity)
	Probably express TLR, Iba-1
Expressing cell surface markers	Antigen presentation
	Chemotaxis
	Facilitates influx of peripheral cells
Cytokine producing	Neuroprotective when release is limited
	Neurotoxic when release is persistent
Neurotrophin producing	Neuroprotective
	Neurosupportive
	Response to insult

indicative of their ability to interact with other cells like T cells [28] (Table 3). Among the most potent activators of microglia is interferon-γ (IFN-γ) which increases expression of MHCII [29–31] and CD40 which enhances the ability of microglia to activate and restimulate T cells [32]. However, Th1 cells also trigger release of inflammatory mediators from microglia indicating that there is bidirectionality of signaling and that interaction between T cells and microglia markedly affects the microenvironment [33]. In addition to promoting interaction with other cells, the evidence suggests that expression of MHCII antigens on microglia [34] and dendritic cells [35] modulates phagocytosis; the finding that MHCII expression colocalizes with CD68, a lysosomal marker of phagocytic activity, in inflammatory conditions, provides further evidence of this [34]. Although MHCII is generally considered to be expressed by microglia rather than other glial cells, there is some evidence that astrocytes also express MHCII (as well as MHCI) albeit to a far lesser extent than microglia [36].

CD86

CD86 and CD80 are transmembrane glycoproteins, which are expressed on antigen-presenting cells including microglia. Interaction of MHCII with T cell receptor, together with activation of CD28 and CTLA-4 by engagement with the costimulatory molecules CD86 and CD80, enables T cell differentiation and activation [37]. CD86 is constitutively expressed as a monomer on the cell surface [37, 38] and preferentially binds to CD28 [39]. Its expression is increased during inflammatory conditions, for example in experimental autoimmune encephalomyelitis (EAE), where it is accompanied by an increase in expression of CD40 and MHCII [40]. Insults, including ischemia, infection, inflammation, and injury, trigger cell activation and several factors which are upregulated in response to insult, or indeed aging or neurodegenerative diseases, trigger upregulation of CD86 and CD80, and their upregulation is generally accompanied by upregulation of MHCII and ICAM [41–43]. Although principally associated with microglia, under some circumstances, CD86, at least, is expressed on astrocytes [44].

Table 2 Proposed cell surface markers of microglial activation

Cell surface marker	Proposed function	Expressed on other cells?
MHCII	Hallmark of activated cells; enables antigen presentation in presence of costimulatory molecules; probably modulates phagocytosis	Macrophages; astrocytes
CD86	Costimulatory molecule; probably required for antigen presentation	Activated astrocytes
CD80	Costimulatory molecule; probably required for antigen presentation	Macrophages
CD11b	Constituitively expressed on microglia; upregulation is indicative of cell activation; plays a role in chemotaxis and modulates leukocyte adhesion and migration	Monocytes
CD40	Marker of activation; contributes to restimulation of T cells; probably affects phagocytosis	Macrophages; endothelial cells; astrocytes
ICAM	Constituitive expression is low but increased upon stimulation; plays a role in cell adhesion	Endothelial cells; macrophages; leukocytes
Iba1	Calcium-binding protein specific to microglia and macrophages; may play a role in phagocytosis	Macrophages
CD200R	Activated by CD200; decreases microglial activation	Cells of the myeloid lineage
Fractalkine R	Activated by fractalkine; decreases microglial activation	Macrophages; leukocytes NK cells; astrocytes; neurons

Table 3 Cell surface molecules on antigen presentation cells, like microglia, interact with cell surface molecules on T cell, modulating cell function

Surface marker on antigen presentation cell (microglia)	Cell surface marker on T cell
MHCII	T cell receptor
CD80 (B1), CD86 (B7)	CD28, CTLA4
ICAM	LFA-1
CD40	CD40L (CD154)

CD11b

CD11b is constitutively expressed on microglia and monocytes but its expression is markedly increased following cell activation [45]. It forms an integrin heterodimer with CD18 to make complement receptor-3 (also called MAC-1), which mediates diverse functions that contribute to its role in mediating inflammatory processes. These include cell motility, cell-mediated cytotoxicity, and chemotaxis [46–48]. It has also been shown to be an important factor in modulating phagocytosis, and it plays a significant role in myelin phagocytosis in multiple sclerosis and EAE [49, 50]. Its expression is upregulated by lipopolysaccharide (LPS) [51] and amyloid β (Aβ) [52] and is increased in a mouse model of AD [53] and following traumatic brain injury [54]. Recent findings from this laboratory have revealed that CD11b messenger ribonucleic acid (mRNA) is increased in the hippocampus and cortex of aged, compared with young, rats (Cowley et al. unpublished) and a similar age-related increase has been reported in the hippocampus of aged mice [55]. Although CD11b is also expressed on monocytes, its upregulation in the brain which occurs in response to insult is considered to be indicative of activation of microglia.

CD40

CD40 is a member of the tumor necrosis factor (TNF) receptor family that is expressed on the surface of immune cells [56] as well as on vascular endothelial cells and astrocytes [57]. It interacts with its ligand, CD154, which is a 39-kDa type II transmembrane protein of the TNF superfamily that is expressed preferentially by activated CD4+ T cells but it is also expressed on other cells [58]. CD40 forms a trimer and, after interaction with its ligand, initiates a complex signaling cascade involving the activation of various kinases including mitogen-activated protein kinases, and transcription factors including nuclear factor κB [57, 59].

The interaction between CD40 receptor and its ligand plays a role in immune cell activation, differentiation, proliferation, and apoptosis; it leads to upregulation of costimulatory molecules such as CD80, CD86, and MHCII as well as production of cytokines such as interleukin-1β (IL-1β), IL-6, and TNF-α [57].

In the brain, microglia express CD40 [32] although expression is relatively low under resting conditions. It is markedly increased upon challenge with LPS and also inflammatory cytokines such as IFN-γ and TNF-α [59–61]. CD154 is known to be expressed on the surface of astrocytes [32] and its expression is upregulated in Alzheimer's disease and in brain injuries [62] but the nature of any interaction between microglia and astrocytes which involves CD40–CD154 interaction is currently unknown. An increase in the expression of CD40 on T cells has been observed in multiple sclerosis (MS), while blocking the interaction between CD40 and CD40L has been demonstrated to be beneficial in a murine model of MS [63].

ICAM

ICAM-1 plays an important role in immune-mediated cell adhesion interactions [64] and intracellular transduction pathways [65, 66]. Several cells, including leukocytes, endothelial cells, and microglia, express ICAM and its expression is low under resting conditions but markedly increased by proinflammatory mediators, primarily due to *de novo* mRNA transcription and translation [67]. Trauma increases endothelial and glial expression of ICAM and also other adhesion molecules such as V-CAM-1 and E selectin [68]. ICAM-1 plays a significant part in adhesion of leukocytes to the endothelium due to its interaction with the integrin LFA-1 [69] and is therefore a factor which contributes to leukocyte infiltration. Predictably, permeability of the blood brain barrier correlates with ICAM expression for example in artherosclerosis, ischemia, and autoimmune disorders [70, 71]. Among the several markers of neuroinflammation is an increase in expression of ICAM which has been correlated with microglial activation in ischemia, following stab injury [72, 73] and in the brain of aged rats [42].

Iba-1

The EF calcium-binding protein, Iba1, derived from the gene *iba1* (ionized calcium binding adapter molecule 1) is a 17-kDa protein which has a restricted expression on cells of the myeloid lineage [74]. In vitro and in vivo analysis confirmed that microglia are the only resident brain cells on

which Iba1 is expressed and an insult, such as facial nerve axotomy which resulted in the morphological changes associated with microglial activation, also resulted in increased expression of Iba1 [75]. The evidence suggests that Iba1 may modulate phagocytic activity because of its ability to modulate cytoskeletal proteins [76].

Microglia Release of Cytokines and Chemokines

Activation of microglia is linked to the secretion of a number of soluble proinflammatory molecules including cytokines, chemokines, eicosanoids, complement, excitatory amino acids, oxidative radicals, and nitric oxide [26]. At high concentrations, these molecules exert detrimental effects on neurons but at low concentrations most are likely to have beneficial effects.

Cytokines

An increase in release of proinflammatory cytokines, such as TNF-α, IL-1β, and IL-6, from microglia, is an indicator of a heightened cell activation state although it is recognized that activated astrocytes also release these cytokines [77, 78]. Perhaps predictably, an increase in expression of these cytokines commonly parallels upregulation of cell activation. For example, an increase in IL-1-positive activated microglia has been reported following head trauma [79] and in Alzheimer's disease [80] while an association between some markers of activated cells and increased expression of IL-1 has been reported in Parkinson's disease [81] and multiple sclerosis [82]. Interestingly, postmortem examination of brains obtained from patients with Parkinson's disease revealed that MHCII-positive microglia in the putamen and substantia nigra were also TNF-α- and IL-6-positive and, in advanced disease, these microglia were associated with neuronal loss [83]. A similar upregulation and/or polymorphism in cytokines like IL-1, TNF-α, and IL-6 has been reported in other conditions which are associated with neuronal degeneration for example epilepsy [84, 85] and cerebral ischemia [86, 87] and also in chronic infections [88, 89]. Interestingly, deficits in cognitive function, ranging from mild and transient, to profound and persistent have been reported in these conditions.

A significant and growing literature, including several reviews, has considered the possible role of microglial activation and the accompanying increase in cytokines particularly IL-1β, in Alzheimer's disease. While there is overwhelming evidence that these changes are evident in the disease state, correlations alone are insufficient to address the question of whether microglial activation is a cause or a consequence of the disease. In brief, the disease is associated with a significant increase in the number of microglia which are clustered around plaques [90]; they do not seem to be phagocytic and their appearance occurs prior to the development of significant neutrophil damage [91]. These microglia are IL-1 immunoreactive; indeed, it has been estimated that, in Alzheimer's disease, there is a sixfold increase in the number of IL-1-immunoreactive microglia [92] and a chronic overexpression of IL-1 is implicated in the initiation and progression of the characteristic neuropathological changes [93].

Increased IL-6 has also been reported in the plasma of patients with Alzheimer's disease [94], while IL-1β, IL-6, IL-12, and TNF-α production by peripheral monocytes under resting conditions and following LPS stimulation was increased in cells prepared from patients compared with controls [95]. While IL-1β has been shown to increase the synthesis [96] and processing [97] of amyloid precursor protein (APP) favoring the release of amyloid peptide fragments, Aβ stimulates microglial activation in vitro [29, 41, 98] and in vivo [29, 99–102] and this is accompanied by increased release of cytokines including IL-1β [29, 100, 102]. These findings suggest that a spiral of damaging events, involving accumulation of Aβ and microglial release of inflammatory molecules, may occur. Clearly, identifying the trigger(s) which initiate this spiral of events is likely to be the key to understanding the pathogenesis of the disease.

While the literature focuses mainly on the negative effects, it is important to consider the findings which have indicated neuroprotective effects of IL-1β. A number of studies have reported that IL-1β stimulates release of nerve growth factor from astrocytes [103, 104] and it has also been shown to be involved in neuronal development [105]. Further evidence of a beneficial effect of IL-1 is indicated by the finding that overexpression of IL-1β in transgenic mice that overexpress APP and PS1 resulted in a reduction in the number of Aβ-containing plaques [106]; while IL-1-receptor-type-1-deficient mice have been shown to exhibit impaired cognitive function, [107] immune-mediated cell-adhesion interactions have been reported.

Chemokines

Chemokines are small, secreted proteins with molecular weights ranging between 8 and 14 kDa. Almost 50 chemokines have now been identified, and their roles in the recruitment and activation of specific leukocytes at sites of inflammation have recently been extended to include a pivotal part in neuroinflammatory processes. In the brain, it is known that they participate in chemotaxis, cell adhesion, and signaling and that expression of several chemokines is markedly altered by inflammatory stimuli [108]. Perhaps the most-studied chemokines in terms of their roles in the

brain are monocyte chemoattractant protein 1 (MCP-1; CCL2), interferon-γ inducible protein 10 (IP-10; CXCL10), macrophage inflammatory protein 1α (MIP-1α; CCL3), and fractalkine (CX_3CL1)

MCP-1 is considered to be a significant factor in driving the acute inflammatory response in the CNS [109–111]. An increase in its expression is a feature of amyotrophic lateral sclerosis and macular degeneration [112] as well as a wide range of other neurodegenerative diseases (see [113]). The evidence suggests that it contributes to CNS injury by stimulating glial production of proinflammatory cytokines [114] and this is supported by the finding that ischemia-induced cytokine expression was markedly reduced in MCP-$1^{-/-}$ mice, [115], while the onset of symptoms in a prion disease model was delayed in these mice [116]. However, proinflammatory cytokines like IL-1β, TNF-α, and IFN-γ stimulate astrocytes to produce MCP-1 [117], triggering a cycle of inflammation. It has been suggested that MCP-1 can be released in response to tissue stress without corelease of cytokines and therefore it may exert actions which are independent of its ability to modulate inflammatory mediators [118]. One hypothesis is that MCP-1 primes glia and therefore decreases the threshold for release of proinflammatory cytokines in response to trauma with the consequent increase in tissue damage and associated neurodegeneration [114].

In addition to its ability to stimulate MCP-1 release from astrocytes, LPS potently stimulates release of another chemokine, MIP-1α from microglia [119] which, with MCP-1, is believed to play a significant role in attracting T cells into the CNS [120]. Neurodegenerative changes are associated with expression of MIP-1α in microglia; for example, a marked increase was observed as early as 8 h after carotid ligation [121] while upregulation of MIP-1 has been documented in multiple sclerosis and the animal models of multiple sclerosis, EAE [122, 123]. Data from this group have established that intracerebroventricular injection of Aβ increases hippocampal expression of MCP-1 [100].

Like most chemokines, basal expression of IP-10 in brain is low but it is markedly increased in both activated astrocytes and microglia [124]. Its expression has been shown to be increased in cortex and hippocampus of middle-aged and aged Tg2576 mice, contrasting with a lack of change in MIP-1α and decrease in fractalkine [125]. Its concentration has been shown to be increased in cerebrospinal fluid of patients with mild (though not advanced) Alzheimer's disease, whereas MCP-1 concentration was increased in all patients with Alzheimer's disease [126]. Interestingly, IP-10-positive astrocytes have been found to be associated with Aβ-containing plaques [127] while its expression is increased following injection of Aβ [100]. Therefore, there is an association between upregulation of IP-10 and neurodegenerative changes, although the evidence indicates that it exerts no significant change alone but acts in combination with other factors to trigger inflammatory change [128].

Microglia and Phagocytic Activity

Like macrophages, microglia are phagocytic cells and are responsible for phagocytosis of cell debris in the CNS. It is assumed that microglia phagocytose Aβ and that this limits plaque formation in Alzheimer's disease and in animal models of Alzheimer's disease [129, 130]. However, recent evidence has suggested that there is an age-related switch in microglial function in the hippocampus of APP × PS1 transgenic mice from a phagocytic function to a more cytotoxic function which coincided with increased expression of Aβ42 [131]. Evidence from Rivest's group has suggested that macrophages are more efficient in terms of their phagocytic activity than microglia in mice that overexpress APP and PS1; this group reported that exposure to irradiation, which decreased bone-marrow-associated macrophages, led to an exaggerated plaque development [132]. These bone-marrow-derived cells were shown to migrate toward Aβ deposits when the plaques reached a certain size and the data indicate that they can eliminate the plaques by phagocytosis both in vivo and in vitro. Resident microglia are present at the onset of plaque formation and it has been suggested that they may play a role early in the disease while blood-derived cells appear at later stage of the disease and may then try to clear the senile plaques [133, 134]. Genetically downregulating Smad2/3 signaling in CD11c-positive cells in C57 mice and crossing these animals with Tg2576 mice has been shown to trigger a marked decrease in Aβ-containing plaques, which was coupled with infiltration into the brain of peripheral macrophages [135]; these data provide further support for a significant role for peripherally-derived phagocytes in clearing Aβ deposits. However, it is not universally accepted that bone-derived macrophages are entirely responsible for phagocytosis of Aβ [136] and, at least in some models, expansion of the resident microglia, rather than infiltration of peripheral cells, accounts for upregulation of cell activation in response to neurodegenerative changes [137]. Recent data from Jucker's laboratory have presented a further complexity; in CD11b-HSVTK transgenic mice crossed with APP/PS1 transgenic mice, treatment with ganciclovir almost obliterated microglia but exerted no appreciable effect on plaque development in spite of a marked increase in macrophage infiltration [138].

A great deal of evidence has been generated in the past 15 or so years which has shown that microglia are capable

of phagocytosing certain forms of Aβ [139–141], but their ability to phagocytose Aβ deposits remains a question of debate. One group reported that injection of soluble Aβ into the brain is cleared after 24 h but fibrillar Aβ is still detected 30 days after injection [142]. While cells were actually capable of phagocytosing fibrillar Aβ, the subsequent processing was such that the fibrillar Aβ was still evident within microglia 30 days later [142]. Degradation of Aβ by microglia appears to be limited by the fact that, although lysosomal proteases are present at high levels, the lysosomal pH is less acidic than in macrophages [143]. It has been suggested that the inability of microglia to completely process fibrillar Aβ may result in activation of cells by the residual Aβ and therefore lead to a persistent neuroinflammatory state. In this context, it is interesting that astrocytes, which respond to Aβ but play no role in clearing Aβ fibrils, release proteoglycans which inhibit microglial phagocytosis [141, 144].

Factors Which Affect Phagocytic Activity

A number of microglial receptors are considered to play a role in modulating phagocytic activity of microglia; these include scavenger receptors, purinergic receptors, Toll-like receptors (TLR), CD40, and triggering receptor expressed on myeloid cells (TREM-2).

Scavenger Receptors

Damaged neurons release nucleotides, particularly adenosine triphosphate (ATP) which attracts microglia to the site of injury. Phagocytosis proceeds following the binding of cell debris to scavenger receptors on microglia; it has been suggested that phagocytosis requires immunoreceptor tyrosine-based activation motif (ITAM)-containing adaptor proteins [145]. However, ATP also serves to activate purinergic receptors, PTX4 and P2Y6, located on microglia, which results in increased phagocytosis [146, 147]. In Wallerian degeneration, two specific receptors, scavenger receptor AI/II and complement receptor-3 (CD11b/CD18 heterodimer), participate in myelin phagocytosis and the evidence suggests that this is phosphatidylinositol-3 kinase dependent [148]. A role for complement receptor-3 in myelin phagocytosis in multiple sclerosis has also been described [49, 50].

Toll-Like Receptors

TLR are a family of transmembrane receptors which respond to molecules that are present in pathogens; the response to these molecules, pathogen-associated molecular patterns, is pivotal in initiating the immune response. Emerging evidence has revealed a role for at least some of the TLRs in the CNS; microglia express TLRs 1–9 [149] and recent evidence indicates that neurons, and perhaps astrocytes, express TLRs 11, 12, and 13 [150, 151]. Particular emphasis has been placed on examining the roles of TLRs 2 and 4 in the CNS and, at least in the case of TLR2, its expression on microglia is markedly increased by insult [152]. Activation of both TLR2 and TLR4 has been shown to stimulate phagocytic activity in murine microglia [153] and BV2 cells [154]. Consistently, a deficiency in TLR2 in APP transgenic mice was associated with an accelerated increase in Aβ formation [155].

Several signaling events appear to modulate TLR-associated phagocytic activity; TLR activation increases expression of receptors which probably play a part in phagocytosis including the scavenger receptor, SR-1, and the Fc receptor, while LPS-induced activation of RAW264.7 cells has been shown to stimulate phagocytic activity in a p38- and Cdc42/Rac-dependent manner [156]. The evidence suggested that activation of p38 was MyD88-dependent but that the alternative pathway was independent of MyD88 [156].

CD40

A few recent reports have identified a role for activation of the CD40 receptor in modulation of phagocytosis. Infusion of human umbilical cord blood cells into PSAPP or Tg2576 mice resulted in reduced Aβ deposition, which suggests an increase in phagocytic activity and this was prevented by a deficiency in CD40, while phagocytic activity of microglia prepared from PSAPP mice treated with these cells was increased [157]. It has also been shown that interaction between CD40 and its ligand, CD154, decreased microglial phagocytosis of Aβ [158].

A role for Notch has also been described; on the one hand, an increase in expression of proinflammatory cytokines is associated with inhibition of Notch transcription in microglia, while activation of Notch by its ligand, Jagged1, decreased proinflammatory cytokine production [159]. Interestingly, this decrease in cytokine production was accompanied by an increase in phagocytic activity, decoupling these aspects of cell activation and providing evidence of separate activation states. However, in a recent review, it was suggested that an "anti-inflammatory microenvironment," was favorable for phagocytic activity, for example, when this aspect of microglial activation is necessary during healing [160]; this may represent an additional "activated state" of microglia.

TREM-2

A number of groups have suggested that TREM-2, which reduces inflammatory responses [161], also plays a role in

phagocytosis [161, 162]. In EAE, beneficial effects were observed in mice treated with bone-marrow-derived and TREM-2-tranduced myeloid precursor cells; in these animals, there was evidence of a reduction in tissue destruction and increased clearance of cell debris [162]. An increase in the newly described soluble form of TREM-2 was detected in the cerebrospinal fluid of patients with multiple sclerosis and the authors suggested that this may act as a decoy inhibiting the activation of the membrane form of TREM-2 [161]. TREM-2 has been identified as a potential target in the search for treatments of multiple sclerosis and is interesting because its activation has been shown to be associated with phagocytic activity and a reduction in proinflammatory cytokine production [163]. Interestingly, TREM-2 is colocalized with Iba-1-positive microglia in association with Aβ deposits in APP23 mice, and its expression is increased in cortical tissue prepared from these mice compared with wild-type mice [164].

Factors Which Modulate Microglial Activation

Microglia respond to insults by altering their morphological and phenotypic states. Multiple factors trigger activation. In vivo, cells respond to insult, including infection, ischemia, inflammation, and mechanical and chemical trauma through multiple molecular mechanisms. In vitro, several factors have been shown to stimulate microglia to express cell surface markers, to release cytokines and chemokines, and to initiate phagocytosis.

IFN-γ

One of the most potent stimuli is IFN-γ which is known to increase expression of cell surface markers [29, 32, 165] and to increase production of proinflammatory cytokines in vivo and in vitro [29, 30, 60, 98, 166]. IFN-γ is a T cell cytokine, primarily released from Th1 cells and natural killer cells, which plays a significant part in modulating growth, maturation, and differentiation of many immune cells [167]. It is a potent activator of microglia and upregulates cell surface markers like CD40 and MHCII, facilitating the interaction of microglia with other cells [60].

While IFN-γ is believed to play a role in neurodegeneration [168, 169], the source of IFN-γ in the brain remains to be clarified. Sporadic reports have suggested that IFN-γ is expressed in different resident cells in the brain but expression levels are low and identified in very particular circumstances. For example, IFN-γ transcripts have been reported in a subpopulation of dorsal root ganglion cells during perinatal and postnatal development [170], in human tumor neuroglia and human astroglial cell lines [171] and in microglia prepared from athymic nude and SCID mice infected with *T. gondii* [172]. In this laboratory, we have been unable to identify IFN-γ in rat neurons, astrocytes, or microglia, though we have shown that there is an age-related increase in IFN-γ concentration in hippocampus [30, 173]. We have recently found that this increase is associated with an increase in infiltration of NK cells into the brain (Murphy et al., unpublished) providing support for the proposal that infiltrating cells which cross when the blood brain barrier is compromised are the source of IFN-γ in the brain [174]. In this context, it is worth noting that blood brain barrier permeability has been shown to be increased with chronic upregulation of IL-1β [175] such as that described in the brain of aged animals [165] and also in aged female rats [176], in 10-month-old mice which overexpress APP [177], and in aged wild-type and $ApoE^{-/-}$ mice [178].

It has been suggested that IFN-γ modulates synthetic processes leading to production of Aβ; although IFN-γ alone does not affect βAPP in a neuroblastoma cell line, it synergizes with TNF-α to increase βAPP mRNA expression and protein synthesis [179]. Similarly, IFN-γ synergizes with TNF-α to increase astrocytic and astrocytoma production of $Aβ_{1–42}$ and $Aβ_{1–40}$ [180].

Aβ

Aβ, like IFN-γ, robustly stimulates microglia to release proinflammatory cytokines. It increases release of IL-1β, IL-6, and TNF-α from cultured microglia, mixed glia, microglial cell lines [29, 181, 182], and monocytes [183]. Aβ synergizes with IFN-γ [184] and LPS [102] to stimulate production of proinflammatory cytokines. In human THP-1 monocytes, the evidence suggests that fibrillar Aβ, but not nonfibrillar Aβ, stimulates release of IL-1β [185]; the authors suggested that the response was triggered by a structural feature of the amyloid fibrils, possibly the cross β-fibril structure [185]. The possibility that microglial activation occurs because of interaction with material specific to the Aβ-containing plaque has also been proposed. Plaques are closely associated with injured or dying cells which secrete nucleotides, complement components, lysosomal enzymes, cellular DNA, and advanced glycation end products; each of these is capable of activating microglia [129, 186–189].

The mechanism by which Aβ induces microglial activation remains to be clarified. Its interaction with several proteins has been described, but it is unclear whether the Aβ-induced microglial activation is mediated by any of these interactions. However, binding of Aβ to a protein complex which includes the α6β1 integrin, CD47, and CD36 results in secretion of inflammatory mediators [190], while interaction with class A scavenger receptors leads to secretion of reactive oxygen species [191]. Aβ also

binds to formyl peptide receptor-like 1 and heparan sulfate proteoglycans on glia, although the consequences of these interactions remain to be identified [192]. Receptors for advanced glycosylated end products [189] and low-density lipoprotein receptor-related protein both bind Aβ and the evidence suggests that these interactions play a role in transporting Aβ across the blood brain barrier [193], while it has been recognized for some time that Aβ binds to the α7 nicotinic acetylcholine receptor with high affinity [194]. Recent evidence has also suggested that some of the actions of Aβ may be mediated by TLR2 [52]. Thus, there are several layers of complexity relating to the effects of Aβ on different cells and unraveling these actions requires a greater understanding of the ways in which Aβ interacts with the array of proteins which appear to have receptor function.

Microglial Function and the Aged Brain

A great deal of evidence indicates that microglial activation is increased in diseased state but it is now accepted that age influences cell activation. Initially, reports suggested evidence of microglial activation in the brain of aged primates [195] but at that time there were few reports, which were somewhat less convincing, which suggested that a similar increase was evident in the rodent brain [196, 197], although cultured cells prepared from brains of aged rats revealed an increased expression of MHCII [198]. Since then, there have been several reports of age-related increases in markers of microglial activation including MHCII, ICAM, and CD86 [42, 165, 173, 182, 199, 200].

Consistent with the view that activated microglia are the primary source of inflammatory cytokines in the brain, there are several reports of age-related increases in expression of IL-1β, TNF-α, and IL-6. Thus, an increase in IL-1-positive microglia has been reported in the brain of aged individuals [201, 202], while increased expression of several inflammatory cytokines including IL-6 and TNF-α has been associated with age-related cortical atrophy [203]. We have consistently reported that IL-1β concentration is increased in the hippocampus of aged rats where it is inversely related to the ability of rats to sustain LTP [173, 199] and, although a correlation between these changes and expression of cell surface markers of microglial activation has been shown, these changes do not always occur simultaneously [204]. Like IL-1β, an increase in IL-6 has also been observed in brain tissue of aged, compared with young, animals [205] and a similar change in TNF-α has been reported in rats [206] and in aged senescence-accelerated mice [207]. These findings, which are indicative of age-related neuroinflammation, are consistent with data obtained from microarray analysis of brain tissue prepared from young and aged animals in which an age-related upregulation of genes involved in the immune response were documented [200, 208].

A number of studies have suggested that decreases in anti-inflammatory cytokine expression accompany the age-related increases in proinflammatory cytokines; thus, hippocampal concentration of IL-4 has been shown to be inversely correlated with IL-1β concentration [165, 209] and, similarly, the age-related increase in IL-6 [210] is accompanied by a decrease in release of the anti-inflammatory cytokine IL-10 from brain slices [211]. While IL-10 suppresses synthesis of proinflammatory cytokines in brain [212], IL-4 decreases IL-1β mRNA synthesis and IL-1β release from glia [165, 213]. IL-4 also attenuates the Aβ-induced increase in markers of microglia, MHC class II, CD86, and ICAM expression and hippocampal concentrations of IL-1β and TNF-α [29, 100].

Interactions Between Microglia and Other Cells

Microglia Interact with Neurons

It is known that microglia interact with other cells and that this interaction modulates their activation state. Recent evidence has shown that microglia are maintained in a quiescent state when CD200 receptor (CD200R) binds its ligand, CD200 [182]. CD200R is a type 1 membrane glycoprotein and the evidence suggests that its expression is relatively restricted to cells of the myeloid lineage; it is highly expressed on macrophages, neutrophils, monocytes, mast cells, and lymphocytes, and, consistent with the functional link between macrophages and microglia, CD200R is also expressed on microglia [182, 214]. In contrast, CD200, which is structurally similar to its receptor [215], is widely expressed on several cell types including neurons and endothelial cells [182, 214].

The proposal that microglia are maintained in a quiescent state by establishing and maintaining an interaction between the CD200 receptor and its ligand is supported by the finding that an activated macrophage/microglial phenotype has been reported in $CD200^{-/-}$ mice in response to stress for example following facial nerve transaction [216], EAE [216], and experimental autoimmune uveoretinitis [217]. Evidence from this laboratory has indicated that there is an inverse relationship between microglial activation (as assessed by cytokine release and expression of cell surface markers of activation) and neuronal expression of CD200 [182, 199]. A decrease in CD200 expression has also been observed in ischemic tissue prepared from rat brain following middle cerebral artery occlusion in which microglial activation has been documented [218] and of particular interest is the recent report that CD200 expression is decreased in postmortem brain tissue obtained from individuals with Alzheimer's disease [219].

In vitro analysis has revealed that LPS-induced or Aβ-induced microglial activation can be attenuated by addition of neurons and this is dependent on CD200–CD200R interaction since the effect of neurons is inhibited when a CD200 antibody is present [182]. Interestingly, the effect of neurons is mimicked by endothelial cells, which also express CD200 [220]. One factor which modulates CD200 expression is the anti-inflammatory cytokine, IL-4; its concentration is decreased with age and is likely to contribute to the coupled age-related decrease in CD200 expression and the age-related increase in microglial activation [182, 199].

It is likely that other interactions between neurons and microglia, in addition to that between CD200 and CD200R, can also influence the activation state of microglia. In their review, Barclay and colleagues suggested that, at least with respect to macrophages, an interaction between CD47 and CD172a, which broadly corresponds to the CD200–CD200R interaction, may occur [214].

We have recently identified that binding of fractalkine to its receptor may also be important in maintaining microglia in a quiescent state [221]. In the brain, fractalkine, a chemokine also known as CX3CL1, is expressed principally on neurons [222, 223] while its receptor, CX3CR1, is expressed mainly on microglia [222]; this complementary distribution, which parallels that of CD200 and CD200R, suggests that the interaction may play a role in modulating microglial activation. Our data and others suggest this is the case; thus, the LPS-induced increase in proinflammatory cytokine production by glia was inhibited by soluble fractalkine [221, 224] and by incubating LPS-stimulated glia with neurons in the presence or absence of an anti-fractalkine antibody [221]. It has also been reported that the neurotoxic effect of activated microglia on cultures of hippocampal neurons was enhanced by the addition of an anti-fractalkine antibody [224], while neuronal cell loss was reported following systemic administration of LPS in CX3CR1-deficient mice and exacerbated neuronal cell loss was observed in a toxin-induced model of Parkinson's disease and in a model of motor neuron disease [225].

Microglia Interact with Astrocytes

While microglia are the main immunomodulatory cells in the CNS, there is little doubt that astrocytes also exert immunomodulatory effects [226] and several of the cell surface markers which are present on microglia that suggest antigen presentation are also expressed on astrocytes [36, 44, 57]. There is also a good deal of evidence suggesting that interaction between these two cell types exist. Both glial cells are highly reactive to their microenvironment; both produce molecules like cytokines and chemokines which are responsible for modulating immune function and both express receptors for several of these molecules [227]. Thus, these cells can exert a powerful influence on each other, facilitating and amplifying immune responses. However, astrocytes also are key producers of neurotrophins and therefore can modulate microglial functions such as cell proliferation, migration, and cell adhesion [228]. Similarly, it has been shown that astrocytes can reduce the potentially damaging effects of microglia. For example, microglial production of IL-12 following stimulation with LPS, or IFN-γ in combination of LPS, is inhibited if microglia are cocultured with astrocytes or with conditioned medium obtained from astrocytes [229]. Similarly, astrocytes have been shown to inhibit LPS-induced changes in inducible nitric oxide synthase expression and NO production in microglia [230]. Recent evidence suggests that one mechanism by which astrocytes modulate microglial activation is by inducing production of the antioxidant enzyme heme oxygenase 1, which subsequently decreases reactive oxygen species production in microglia [231] although a role for transforming growth factor-β has also been identified [232].

A modulatory effect of astrocytes on the neurotoxic effects of HIV-1-infected microglia has also been described which illustrates the protective effect of these cells. Neuronal cell death was examined after incubation in the presence of conditioned medium obtained from HIV-1-infected microglia, astrocytes, or infected microglia co-cultured with astrocytes; a tenfold increase in cell death occurred when neurons were incubated in conditioned medium obtained from microglia and this was markedly reduced by incubation of cells in conditioned medium obtained from microglia co-cultured with astrocytes [233].

Microglia Interact with T Cells

In addition to the interaction of microglia with neurons and astrocytes, it is known that they also interact with T cells. On the basis of data obtained from in vitro analysis by a number of groups, it can be concluded that Th1 cells lead to upregulation of cell surface markers on microglia and increased production of proinflammatory cytokines. Thus, Aloisi and colleagues found that Th1 cells enhanced expression of MHC class II, CD40, and CD54 molecules on microglia [33, 120], while incubation of microglia in the presence of the myelin basic protein-specific Th1 cells also induced an increase in cell surface expression of MHC class II, CD80, CD86, CD40, and CD54 [234] and increased secretion of cytokines including TNF-α and IL-6 [234, 235]. A further study using human tissue provided additional evidence of a T cell–microglial interaction. Incubation of U937 cells or microglia derived from fetal human brains with anti-CD3-stimulated human T lympho-

cytes resulted in a marked increase in TNF-α in the conditioned medium; this was not observed when untreated lymphocytes were used or when T cells were pretreated with minocycline, which was shown to mediate its effect by decreasing CD40L expression [236].

The issue of whether cell–cell contact is required or whether release of soluble factors from T cell is responsible for mediating the effect is not absolutely clear; on the one hand, exposure of microglia to IFN-γ mimics the effect of Th1 cells [120], although others have suggested that soluble factors exert minimal effect on microglial activation [235]. It was concluded by Seguin and colleagues that cell–cell interaction is important since the effect of T cells was not blocked by anti-IFN-γ antibody [234]. In contrast to the effects of Th1 cells, Th2 cells exerted no appreciable effect on cell surface markers or cytokine and chemokine production and they did not inhibit the Th1-induced changes [120, 234] and supernatant prepared from Th2 cells did not alter microglial expression of cell surface markers or release of cytokines or chemokines [234].

The study of this interaction between T cells and microglia in vivo has advanced, to a significant extent, by assessing communication between the cells in EAE. In this model, peripherally primed $CD4^+$ T cells cross the blood brain barrier and influence the activation state of microglia but it is important to note that, when T cells enter the CNS, their activation and polarization are influenced by the CNS microenvironment. It has been known for some time that cell surface expression of MHCII and the adhesion and costimulatory molecules CD54, CD40, CD80, and CD86 on microglia, which is indicative of their role in antigen presentation, is increased in multiple sclerosis or EAE [237, 238]. This profile suggests that these cells interact with infiltrating autoimmune T cells and a particular role for CD40 has been identified. CD40 ligand-positive T cells have been identified in the postmortem brain of MS patients and these cells were colocalized with CD40-positive cells in active lesions, while administration of an anti-CD40 antibody prevented the symptoms of EAE [63]. Mapping the progression of symptoms in EAE, it was established that the initial changes in microglial activation were independent of CD40 but that subsequent activation, as well as leukocyte infiltration and proliferation of encephalitogenic T cells, did not occur in bone marrow chimera mice with CD40-deficient microglia [40]. It is interesting that fewer T cells and peripheral macrophages accumulated in the CNS when CD40 expression on microglia was decreased.

Concluding Remarks

Although a great deal of literature describes the detrimental role of activated microglia, it is known that they also contribute to neuroprotection [130, 132, 239–241]. Indeed the process of cell activation derives from the need to provide protection from inappropriate stimuli and to provide trophic support for neuronal function especially during development [242, 243]. During this time also, the phagocytic activity of microglia is important for elimination of cell debris derived from apoptotic cells. This appears to rely on the interaction between the phosphatidylserine-expressing apoptotic cell and the phosphatidylserine receptor which is expressed on microglial cells [244, 245]. In short, controlled activation of microglia in response to stressors probably plays an important role in maintaining and restoring homeostasis while prolonged extensive activation can be detrimental to cells as a consequence of release of inflammatory mediators and neurotoxins. Indeed, studies in vivo and in vitro have suggested that the neuroprotective or neurotoxic effects of microglia on neurons might depend on the nature of the stimulus, the molecules released, and the cell-to-cell interactions between microglia and neurons.

This review started by suggesting that microglia are probably never “resting” since the role of the ramified cell is to sample the microenvironment by means of continuously active, motile processes and to initiate a response when a threat is perceived. The issue is how to evaluate microglial activation and differentiate between responses which are protective or detrimental to cells. At the very least, cells can adopt one of several states. (a) Microglia can express cell surface markers indicating that they will interact with other cells; one well-accepted consequence of this is that microglia act as antigen-presenting cells and interact mainly with T cells. However, microglia interact with other cells and cell surface markers other than those involved in antigen-presenting function are expressed; examples include CD200 and fractalkine receptors. (b) Microglia release chemokines and therefore play a role in chemotaxis. (c) Microglia can retract their processes and become motile, reflecting the fact that they can move to a site of injury or inflammation to exert the appropriate action. (d) Microglia release cytokines, the role of which may be protective or damaging. (e) Microglia release reactive oxygen and nitrogen species, which are generally considered to be detrimental to cells. (f) Microglia can adopt an ameboid morphology, which is thought to reflect the transition to a phagocytic phenotype. It remains to be clarified whether microglia can adopt a state which is described simply by one of these phenotypes or whether cells adopt multifaceted and additional or perhaps transitional states. At present, we also do not know whether specific stimuli trigger particular phenotypic states and an important outstanding question is what factors modulate the appropriate molecular switches to ensure that the activated state persists or not. Until these questions are resolved, it is

difficult to envisage that potential therapeutic advances, based on modulation of microglial function, can be made.

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