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INVITED REVIEW

Microglia Biology in Health and Disease

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Abstract Microglia cells are resident central nervous system (CNS) leukocytes that regulate innate immunity and participate in adaptive immune responses in CNS tissue. However, microglia cells also appear to play an important role during normal function of the mature nervous system. In response to injury, ischemia, and inflammatory stimuli, microglia cells assume an activated phenotype associated with proliferation, migration to the site of injury, phagocytosis of cellular debris, and elaboration (Power and Proudfoot 2001) of both neurotoxic and neurotrophic factors. Recent reports strongly suggest that regulating microglia function may be a fruitful future therapeutic target for the prevention of neurological dysfunction in a variety of CNS injuries and chronic diseases. Thus, developing a thorough understanding of extracellular signals that activate microglia as well as a complete catalogue of microglia responses to activating stimuli in both the healthy and diseased state are crucial scientific endeavors. This review presents the current understanding of the biology of microglia during normal CNS function as well as in response to CNS injury or neurodegenerative disease. In addition, microglia modulate both the activation and down-regulation of the adaptive immune response in the CNS. Evidence that microglia cells play a primary role in regulating CNS immune responses will also be discussed.

Key words microglia · neuroinflammation · cytokine · chemokine · prostaglandin · phagocytosis · neurotrophic factor · ATP

Introduction

Microglia are the resident immune cell population of the central nervous system (CNS; Kreutzberg 1996). First described as "the third element" of the central nervous system by Cajal (1913), this definition applied to all cells that were morphologically different from neurons (first element) and astrocytes (second element). Whereas this definition obviously also included oligodendrocytes, Cajal's disciple, Rio-Hortega (1921), formally described these two cells as separate entities and provided the first systematic investigation of microglial cells (Rio-Hortega 1932). Much time has passed since these early descriptions, yet the lack of specific markers to unambiguously identify microglial cells hampered progress considerably. With the advent of monoclonal antibodies and improvements in mammalian cell culture, the last two decades brought enormous insight into the central role of microglia in many acute and chronic neurological diseases, such as stroke, Alzheimer's disease (AD), or multiple sclerosis (Carson 2002; Eikelenboom et al. 2002; Streit 2002; Danton and Dietrich 2003; van Rossum and Hanisch 2004). In the adult, healthy brain microglia are found as so-called "resting" microglia, characterized by a small cell body with fine, ramified processes and low expression of surface antigens. CNS injury triggers rapid activation of microglial cells. These activated microglia participate in the pathogenesis of neurological disorders by secreting various inflammatory molecules such as cytokines or nitric oxide (NO) (Hanisch 2002). When CNS cells die, microglia can be further activated and become phagocytes (Streit 2002). The wide array of biological changes that develop in activated microglia establishes an intricate network of communication between additional cellular arms of the CNS immune response. For example, microglia secrete cytokines and prostaglandins that signal to astrocytes amplifying their inflammatory response and resulting in even more injurious accumulation of neurotoxins in CNS tissue (van Rossum and Hanisch 2004). Microglia activation also results in the release of inflammatory mediators that recruit more microglia to the site of activation as well as promote the infiltration of immunomodulatory cells from the peripheral blood. When the activating stimulus wanes, microglia participate in the down-modulation of the immune response and can regulate their own apoptosis via the secretion of anti-inflammatory cytokines. This remarkable ability to rapidly respond to a changing external environment and direct the

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response of the rest of the innate and adaptive immune system in the CNS make microglia a prime target for therapeutic intervention in a wide variety of CNS insults.

Normal microglia function

While recent years have considerably advanced our understanding of activated microglia in the pathogenesis of neurological diseases, still, very little is known about the role of resting microglial cells in the unperturbed CNS. This is mainly because of the capricious nature of the object under study. Most of our knowledge about signals leading to microglial activation is derived from *in vitro* studies. However, to culture microglial cells, one has to inevitably remove them from the tissue they are protecting, typically using proteolytic and mechanical means of dissociating cells from CNS tissue. This of course leads to microglial activation, an unfortunate conundrum. Histological approaches to study microglial cells, on the other hand, preserve microglial cells in their tissue context, although they only allow for a freeze-frame picture at the point the brain was placed in fixative. Only very recently, *ex vivo* flow cytometry and multiphoton confocal *in vivo* imaging shed some light on microglial physiology *in situ*. In elegant experiments, two groups using GFP expressing microglial cells reported independently that microglial cells patrol the CNS (Davalos et al. 2005; Fetler and Amigorena 2005; Nimmerjahn et al. 2005). On the time scale of minutes, the fine, elaborate microglial processes constantly reorganize and appear to probe the neuronal microenvironment in the unperturbed CNS. Whereas the signals responsible for this constant remodeling of microglial cytoarchitecture remain obscure, the mere fact of such rapid morphological changes was completely unanticipated. One small caveat to these studies is the animal model used for these experiments. In both studies, microglial cells express GFP by the virtue of a knock-in–knockout approach originally aimed at disrupting CX_3CR1, the fractalkine receptor (Jung et al. 2000). Although both studies used heterozygous animals, the disruption of one genomic copy of CX_3CR1 and therefore reduction of CX_3CR1 expression might have influenced microglial motility (Harrison et al. 1998; Maciejewski-Lenoir et al. 1999). Nevertheless, the idea that microglia cell morphology is constantly shifting in the normal CNS is a quite revolutionary concept, and further studies are underway to address the lingering questions.

Signals for microglia activation

It is widely believed that substances released from damaged CNS cells trigger microglial activation, consequently leading to long-term changes in gene expression and reorganization of the cell phenotype (Kreutzberg 1996; Streit 2002). While most of our understanding of microglial activation derives from *in vitro* experiments, the aforementioned *in vivo* imaging experiments also shed some light on possible microglial activating signals in the tissue context. High-energy laser pulse-induced injury of CNS tissue or parenchymal blood vessels triggered immediate and massive morphological responses of microglial cells in close proximity (Davalos et al. 2005; Nimmerjahn et al. 2005). The damage of blood vessels (Nimmerjahn et al. 2005) could lead to spill of a multitude of blood-derived factors into the CNS parenchyma serving as microglial activating factors (Möller 2002; Möller et al. 2006). The response to CNS tissue injury (Davalos et al. 2005), however, was inhibited by blockade of ATP signaling, and application of ATP led to similar morphological changes as laser-induced CNS injury. This unquestionably implicates ATP as a mediator of early microglia morphological changes. However, whether ATP is also the predominant activator for long-term physiological changes in microglial cells remains to be determined. Both experimental approaches only lasted for mere hours. This leaves open the possibility that other, slower acting factors still play a role in the reorganization of the microglial phenotype seen in acute and chronic neurological disease after days and weeks.

This leads to the question of how microglial cells detect changes in their environment. Not surprisingly, they express a large number of cell surface and nuclear receptors that play a critical role in initiating and/or modulating their immune response (Hanisch 2002; Möller 2002; van Rossum and Hanisch 2004). These include, but are not limited to, receptors for factors, such as complement, immunoglobulins, cell adhesion molecules, steroids, bacterial products, misfolded proteins, and last, but not least, cytokines and chemokines. The number of receptors described is continuously increasing and cannot be covered here in detail. Several recent reviews, however, address these specific areas in considerable detail (Hanisch 2002; Husemann et al. 2002; Möller 2002; Rogers et al. 2002; van Rossum and Hanisch 2004; Inoue 2006). Here we will exemplify the concept of receptor-mediated microglial activation with a few selected receptors for bacterial products, pathological proteins, ATP, and serum factors.

Microbial signals

The activation of microglial cells by lipopolysaccharide (LPS, endotoxin) was one of the first to be described in the literature (Hetier et al. 1988). Like all myeloid cells, microglial cells respond strongly to an LPS challenge with the release of a multitude of cytokines, chemokines, NO, and proteases (see below). These effects are mediated by TLR4, a member of the toll-like receptor (TLR) family (Takeda et al. 2003). The TLRs are a family of pattern-recognition receptors involved in detecting microbial infection and currently include 11 cloned receptors (TLR1–TLR11). The TLRs respond to a variety of different stimuli including lipoteichoic acid (TLR2), LPS (TLR4), flagellin (TLR5), single-stranded viral RNA (TLR7), and unmethylated CpG DNA of bacteria and vi-

ruses (TLR9; Takeda et al. 2003). Microglial cells express TLR1–TLR9 (not TLR10, TLR11 not tested), which renders them sensitive to almost any type of bacterial and viral challenge. Of these stimuli, microglial activation by LPS/TLR4 is by far the best described (Rivest 2003). Because it is triggering microglial responses for most parameters investigated (e.g., proliferation, migration, NO, and cytokine release), it is frequently used as a positive control in *in vitro* and *in vivo* experiments. Downstream of TLR4 activation is a signaling cascade involving MyD88, IRAK, ERK, p38, and JNK kinase activation finally leading to transcriptional regulation via NF-κb (Rivest 2003; Takeda et al. 2003). Viral pathogens are much more commonly capable of invading neural tissues than bacteria. However, with the exception of human immunodeficiency virus (HIV), which has been extensively studied and independently reviewed (see Berman et al. in this issue), the specific signaling pathways elicited by viral pathogens in microglia are only beginning to be elucidated. Microglia have been demonstrated to respond to CNS infection with herpes simplex virus (HSV-1; Esiri et al. 1995). Experiments using TLR2 knockout mice support the hypothesis that neural injury in HSV-1 encephalitis may be downstream of TLR2-mediated proinflammatory signaling (Aravalli et al. 2005). Another herpes virus, cytomegalovirus, has been shown to stimulate microglial production of antiviral cytokines (Cheeran et al. 2001) and the T-cell chemoattractant chemokine CXCL10 (IP-10). CXCL10 synthesis is dependent on activation of microglia p38 MAP kinase that interestingly can be suppressed by virally expressed interleukin 10 synthesized by infected astrocytes (Cheeran et al. 2003). For other identified viral pathogens of the human CNS including varicella zoster, measles, West Nile, and enteroviruses, there are no reports identifying a specific molecular signaling pathway that promotes microglia responses to these viruses.

Pathological proteins

In neurodegenerative diseases associated with a proteinopathy, such as AD, Huntington's disease (HD), or the familial form of amyotrophic lateral sclerosis (ALS), the observed microglia activation prompts the question whether microglial cells are activated by abnormal protein. The best-documented case so far is AD. β-Amyloid (Aβ), which is the building block of the hallmark plaques of AD, has been shown to be a potent activator of microglial cells (Rogers et al. 2002). Although responses to Aβ, such as microglial proliferation and cytokine release, have been well documented, there is still some controversy about the receptor(s) triggering these effects. There is good evidence that scavenger receptors (SR) play an important role (Husemann et al. 2002). However, other data suggest the involvement of formyl peptide receptor-like 1, receptor for advanced glycosylation end products, a complex involving CD36, alpha(6)beta(1)-integrin and CD47, or the Serpin–enzyme complex receptor (Verdier et al. 2004). Final judgment is further complicated by the fact that different preparations of Aβ (nonaggregated *vs*. fibrillary) trigger different effects and might be mediated via disparate receptors. In addition, it appears that costimulation by additional inflammatory signals acting at the CD40 (Townsend et al. 2005) receptor or the prostaglandin EP2 (Shie et al. 2005a) receptor strongly influences the manner by which microglia respond to Aβ.

Extracellular ATP

Microglial activation by ATP has been known for almost 15 years (Kettenmann et al. 1993). The initial electrophysiological experiments showed an ATP-induced cation current. In the following years, a considerable amount of data established ATP as a major activator of microglial cells (Inoue 2006). The effects for ATP are mediated by P2 receptors, which are encoded by two distinct gene families. The P2X receptors are ligand-gated ion channels, whereas P2Y receptors are metabotropic G-protein-coupled receptors (Burnstock 2004). Microglia express both P2X and P2Y receptors (Brautigam et al. 2005). The functional expression of these receptors is regulated during microglial activation (Möller et al. 2000), and P2 receptor activation triggers such diverse effects as $[Ca^{2+}]_i$ signals, proliferation, and cytokine release (Inoue 2006) as well as microglial activation after CNS injury *in vivo* (Davalos et al. 2005). Although the involvement of specific P2 receptor subtypes for most reported effects remains vague, experiments with P2 receptor knockout animals shed light on the role of some P2 receptors in microglial cells. For example, the expression of P2X4 is enhanced in spinal microglia after peripheral nerve injury, whereas genetic ablation and pharmacological blockade of P2X4 receptors significantly reduced the level of the neuropathic pain in an animal model (Inoue 2006).

Serum factors

Serum contains a number of factors involved in the response to vascular injury, which have physiologic impact on microglia when they come in contact with the CNS parenchyma. After breakdown of the blood–brain barrier, a virtually unlimited pool of serum factors inundates the CNS parenchyma and serves as activation signals for microglial cells. Consequently, microglial cells have been shown to respond to serum factors including but not limited to lysophosphatidic acid (Möller et al. 2001), sphingosine-1-phosphate (Tham et al. 2003), immunoglobulins (Stangel and Compston 2001), complement (Möller et al. 1997; McGeer and McGeer 2002), and thrombin (Weinstein et al. 2005). The effects are mediated by dedicated G-protein-coupled receptors and trigger responses spanning from proliferation and migration to $[Ca^{2+}]_i$ signals and cytokine release. However, recent experiments with thrombin documented the difficulty of working with serum-derived products (Hanisch et al. 2004; Weinstein et al. 2005). These reports showed that plasma thrombins are contaminated with other unknown plasma-derived products, which contribute

partially to the signals reported. This, of course, does not invalidate serum factors as microglial activation signals, after all the products are serum-derived. However, it demonstrates the necessity of careful controls when investigating the activation of microglial cells, as these cells express a multitude of (as yet unidentified) receptors, and contaminants in reagents might interact with microglia at high potency.

Biological responses in activated microglia

Migration

One of the earliest responses of microglia to an activating physiological stimulus is migration to the site of injury or inflammation. Injury to CNS tissues results in the release of chemotactic factors that stimulate microglia migration to the site of neural injury. Many of the promigratory factors are members of the chemokine family of chemotactic molecules. Microglia have been demonstrated to move along chemokine gradients *in vitro* and appear to be stimulated to move into the developing CNS via the same chemotactic gradients observed in injury models (Cartier et al. 2005). Extracellular ATP and ADP released from ischemic and traumatic CNS injuries also stimulate microglia migration (Honda et al. 2001). Microglia also migrate toward some trophic factors such as vascular endothelial growth factor (Forstreuter et al. 2002). In addition to soluble factors that can generate concentration gradients that promote and direct microglia migration, changes in the extracellular matrix of injured or diseased CNS tissue may also regulate microglia migration. For example, microglia require the β2-integrin CD11a for normal migration in response to injury (Ullrich et al. 2001). Another matrix protein, tenascin-R, is antiadhesive for activated microglia both *in vitro* and on tissue slices, suggesting that its presence inhibits microglia migration. In response to axotomy, exposure to TNFα, or treatment with microglia-conditioned medium, tenascin-R expression is down-regulated, allowing microglia migration into injured or inflamed regions of the CNS (Angelov et al. 1998).

Proliferation

Microglia cells are capable of entering the cell cycle in response to a variety of stimuli. Traumatic and ischemic lesions, neoplasms, and regions of neuronal or axonal degeneration expose microglia to strong signals for proliferation. As resident microglia invade an injured region of the CNS in response to chemokine gradients and changes in the ECM, they elaborate cytokines that stimulate microglia division including interleukin (IL)-1β, IL-4, and interferon gamma (IFNγ; Kim and de Vellis 2005). The most potent microglia mitogenic factors *in vitro* are the colony-stimulating factors (CSF) macrophage (M-CSF) that can be secreted from activated astrocytes (Kim and de Vellis 2005) and granulocyte macrophage (GM-CSF) that acts in conjunction with the CD45 tyrosine kinase to stimulate microglia proliferation (Suh et al. 2005). In addition, neurotrophic factors such as BDNF and NT-3 are released by activated microglia and act in a paracrine fashion as microglia mitogens (Elkabes et al. 1996). Interestingly, bone marrow transplantation methods have shown that resident microglia, rather than invading macrophages, make up the proliferative pool of microglia, and that these dividing microglia express the stem-cell marker CD34 (Ladeby et al. 2005). In fact, it has been suggested that resting microglia are more like an undifferentiated progenitor cell population than typical tissue macrophages, with the capability to divide and differentiate into a number of different phenotypes producing a heterogeneous population of activated microglia cells (Santambrogio et al. 2001). Indeed, each of the microglia functions discussed below likely develops in a particular subset of microglia *in vivo*.

Nitric oxide production and the respiratory burst

Cells of the monocyte lineage are often the first line of defense against an invading pathogen. The armamentarium from which they can attempt to derail pathogen attempts at infection and reproduction includes the ability to generate NO and produce a respiratory burst. Both peroxinitrate, a byproduct of NO, and the superoxide generated by the respiratory burst produce oxidative DNA damage to nearby pathogenic organisms. However, microglia activation secondary to noninfectious injury has the unfortunate side effect of releasing these toxic reactive oxygen species into the neural environment leading to oxidative injury. In microglia, NO is generated by the inducible isoform of NO synthase (iNOS or NOS-2). Many discreet stimuli for microglia activation as well as a wide variety of neurological injury or disease models are associated with induction of microglia iNOS and the generation of NO. Cultured human microglia may be different from standard rodent models in that they do not appear to express iNOS or make NO, whereas cultured human astrocytes are capable producers of iNOS and NO (Kim and de Vellis 2005). However, human microglia cultures generated from fetal tissue may not be representative of microglia biology in the more mature CNS. Evidence from pathologic evaluation of MS lesions demonstrates that iNOS is expressed by a large portion of the cells labeled with microglia markers in both the parenchyma and perivascular space, whereas GFAP-labeled astrocytes only occasionally expressed iNOS near the edge of the active inflammatory lesion (Hill et al. 2004). Cells of the monomyeloid lineage can also generate toxic ROS aimed at disabling or killing microorganisms by generating a respiratory burst. The respiratory burst produces superoxide anion and hydrogen peroxide via the enzyme NADPH oxidase. Cultured microglia cells express NADPH oxidase (Sankarapandi et al. 1998). The catalytic subunit of the enzyme also colocalizes with microglia markers in tissue sections and is up-regulated in response

to injury (Green et al. 2001). However, the amount of superoxide generated by cultured microglia is 20–40 times lower than that generated by neutrophils (Sankarapandi et al. 1998). Nevertheless, animals deficient in NADPH oxidase activity are protected from ischemic stroke (Walder et al. 1997) and *in vivo* and *in vitro* models of Parkinson's disease (Qin et al. 2004).

Phagocytosis

Microglia are the predominant phagocyte in the CNS. They will engulf microbes and can present engulfed proteins as antigen to T cells that traffic in and out of the CNS (see below) to stimulate an adaptive immune response. Microglia also engulf pathological proteins such as Aβ, and modulation of Aβ phagocytosis by microglia is under intensive study as a potential therapeutic intervention in AD (Rogers et al. 2002). In addition to surveillance of the CNS for microbes and pathological proteins, phagocytosis by microglia is the main means for removal of apoptotic cells and cellular debris in the CNS. How microglia recognize cellular elements as appropriate for phagocytosis has not been completely determined, but several important signals have been identified. These include the expression of phosphatidylserine on the surface of apoptotic cells and the interactions with the microglia vitronectin receptor and the CD36 scavenger receptor (Witting et al. 2000; Stolzing and Grune 2004). Another important signal for microglia phagocytosis is the triggering receptor expressed on myeloid cells-2 (TREM-2), an orphan receptor that appears to bind to a wide variety of microbes. Activation of TREM-2 promotes microglia phagocytosis and inhibits the expression of proinflammatory cytokines, whereas deficiency of TREM-2 impairs clearance of apoptotic neurons and promotes differentiation of microglia into a cytokine-producing phenotype (Takahashi et al. 2005). Humans deficient in TREM-2 (Nasu–Hakola disease) develop a progressive frontotemporal dementia that begins in the third or fourth decade of life, suggesting that TREM-2-mediated differentiation of microglia into phagocytes is required for normal maintenance of the adult human CNS. It is not currently understood whether the requirement for TREM-2 stems from the need to prevent the cytokine-secreting function of microglia or to promote phagocytosis that not only may be required for removal of debris from the CNS but may also be important for the immunomodulatory events that proceed after microglia engulf microbes or other apoptotic leukocytes. For example, when microglia phagocytose apoptotic T cells, the secretion of IL-12, interferon-γ (IFNγ), and tumor necrosis factor-α (TNFα) is diminished, and their ability to stimulate T-cell expansion is significantly reduced (Magnus et al. 2001). Interestingly, one of the agents commonly employed as immunomodulatory therapy for MS, IFNβ, enhances the ability of microglia to phagocytose apoptotic T cells, thereby resulting in a suppression of the immune response (Chan et al. 2003). This finding suggests yet another mechanism of action by which IFNβ may influence disease progression in MS.

Antigen presentation

To generate an adaptive immune response, antigen must be presented to T cells by antigen-presenting cells (APCs) that engulf antigen-containing microbes, infected cells, or other foreign materials, process antigen in vacuoles, and present the processed peptides on the cell surface to interact with T cells. Professional APCs, known as dendritic cells, can be identified by expression of specific surface markers such as class II major histocompatibility complex (MHC-II) and CD11c. In response to specific extracellular signals, a subset of microglia will up-regulate expression of dendritic cell markers, costimulatory molecules such as B7.1 and B7.2, and the cathepsin protease required for generation of antigen peptides (Aloisi et al. 2000). Following CNS infection or the experimental autoimmune encephalomyelitis murine model of multiple sclerosis, CNS cells can be isolated that concurrently bear some features of recently divided microglia and are capable of presenting antigen to T cells (Fischer and Reichmann 2001). Studies of tissue of additional types of injury and disease including stroke (Reichmann et al. 2002), ALS (Henkel et al. 2004), and neoplasms (Watters et al. 2005) suggest that based on expression of surface markers, microglia may be a source of APCs in a variety of CNS pathological conditions. Despite the presence of appropriate surface antigens and the ability to stimulate T cells *in vitro*, it has been debated whether APCs in the CNS are actually dendritic cells that invade from peripheral blood or differentiate from the available pool of resting microglia. Experiments employing bone marrow chimeric mice elegantly demonstrated that both peripheral blood and parenchymal microglia must serve as APCs to observe the neuroprotective function of activated T-helper cells (Byram et al. 2004). Thus, microglia appear to play an important role as APCs even if they may require some additional signals from circulating dendritic cells to generate a complete response.

Secretion of diffusible factors

One of the major functions of microglia is to send signals to other cells that will regulate the inflammatory response following exposure to a specific insult or infection. Four major classes of molecules are responsible for communicating signals from microglia to surrounding cells and invading leukocytes. These include cytokines, chemokines, trophic factors, and small molecule mediators of inflammation such as prostaglandins.

(1) Cytokines are immunomodulatory peptides that include interleukins, IFNs, TNFα, and TGFβ. Cultured human microglia express mRNA for IL-1α/β, IL-6, IL-10, IL-12, IL-15, and TNFα (Kim and de Vellis 2005), whereas additional studies have documented expression of IL-3, IL-18, IFNs, and TNFα in rodent

microglia (Hanisch 2002). Microglia activation generally increases cytokine expression, and proinflammatory cytokines are the first to be released and tend to have both toxic effects on surrounding cells as well as CNS effects that are separate from their role in stimulating the immune response. For example, sickness behavior with fever and decreased caloric intake is mediated by cytokines (Konsman et al. 2002). Microglia also elaborate receptors for most of these cytokines resulting in autocrine feedback loops that are likely to be crucial for the eventual down-regulation of an inflammatory response. Anti-inflammatory cytokines IL-4/IL-10/IL-13 and TGFβ may not be constitutively expressed or available for release until the proinflammatory response is well underway. Generally, the anti-inflammatory cytokines have neuroprotective properties (Hanisch 2002). Both IL-4 and IL-13 appear to support eventual microglia apoptosis (Yang et al. 2002) days to weeks following a discreet inflammatory stimulus and have been demonstrated to act as an important feedback mechanism to eventually turn off an inflammatory response *in vivo* (Shin et al. 2004).

(2) Chemokines are a family of peptide chemoattractant molecules that interact with a specific family of G-protein-coupled receptors. Cultured microglia exposed to bacterial antigens, cytokines, or Aβ will express and release a number of chemokines including but not limited to KC, MIP-1α, MIP-1β, MIP-2, MCP-1, RANTES, IP-10, and IL-8 (Hanisch 2002). Chemokine names have been modified to incorporate a systematic strategy based on amino acid sequence. Previously, chemokines had been classified as α, β, γ, or δ chemokines based on the position of the first two cysteines. Now the classification system has been incorporated into the name so that all chemokine (and chemokine receptor) names begin with C, CC, CXC, or CX3C, followed by L for ligand or R for receptor and then a numbered designation (see Table 1). Microglia also express receptors for many of the microglia-elaborated chemokines, suggesting that one of the main functions of chemokine release is to attract additional microglia to the site of the insult. This assertion may be difficult to prove using *in vitro* methods because cultured microglia have very low level of surface receptor expression of the CXCR4 and CCR5 chemokine receptors compared to microglia cultured with neurons (Garden et al. 2004). Using an alternate approach, it was demonstrated that chronic exposure to MCP-1 via transgenic over-expression led to chronic microglia activation (Takahashi et al. 2005). One very interesting chemokine, known as fractalkine, is constitutively made by healthy neurons and actually appears to have an anti-inflammatory effect on microglia (Zujovic et al. 2000). The chemokine stromal cell-derived factor 1 appears to act on multiple CNS cells, including neurons and microglia (Meucci et al. 1998; Kaul and Lipton 1999), and promotes synergistic release of TNFα from astrocytes and microglia (Bezzi et al. 2001). Thus, despite the finding that many chemokines are released concurrently during microglia activation, individual chemokines may induce very specific responses in a particular cellular or cytokine context.

(3) Trophic factors that promote neuronal survival are frequently synthesized in and released from microglia. Classical neurotrophins, including NGF, BDNF, and NT-3, basic fibroblast growth factor, and glial-derived neurotrophic factor (GDNF) are synthesized by microglia (Presta et al. 1995; Elkabes et al. 1996; Honda et al. 1999). GDNF is also expressed by microglia and, in conjunction with BDNF, may be responsible for guiding regenerating axons toward a lesion (Batchelor et al. 2002). In addition to providing trophic support to surrounding neurons and neuronal processes, trophic factors from microglia may also act in an autocrine fashion to regulate the ability of microglia to sustain the proinflammatory state. For example, NGF treatment causes a reduction in MHC class II expression on microglia (Neumann et al. 1998).

Table 1 Microglia chemokines and their receptors

CC name	Prior name	Receptor(s)	Expression pair	Reference
CCL2	MCP-1	CCR2	Yes	(Galasso et al. 2000; Simpson et al. 2000)
CCL3	MIP − 1_{α}	CCR1/5	Yes	(Simpson et al. 2000; Power and Proudfoot 2001)
CCL4	MIP − 1_{β}	CCR1/5/8	Yes	(Simpson et al. 2000; Power and Proudfoot 2001)
CCL5	RANTES	CCR1/3/5	Yes	(Albright et al. 1999)
CCL19	MIP-3rβ	CCR7		(Columba-Cabezas et al. 2003)
CCL22	MDC	CCR4		(Columba-Cabezas et al. 2002)
CXCL1	GROα/KC	CXCR2	Yes	(Janabi et al. 1999)
CXCL8	IL-8	CXCR1/2	Yes	(Flynn et al. 2003)
CXCL10	IP-10	CXCR3		(Flynn et al. 2003)
$Cx3CL_1$	Fractalkine	CX_3CR1	Yes	(Harrison et al. 1998)

Chemokines generated by microglia are listed in the far left column. Expression pairs are those chemokines for which at least one receptor is also expressed in microglia.

(4) Activated microglia release small molecule lipid inflammatory mediators including arachidonic acid, prostaglandins D_2, E_2 and $F_{2\alpha}$, platelet-activating factor, thromboxane B_2, and leukotriene B_4 (Minghetti and Levi 1998). Arachidonic acid released by phospholipase activity is the substrate for generation of these metabolites of lipoxygenase and cyclooxygenase activities. Microglia appear to be an important source of prostaglandins in the CNS, secreting significantly more prostanoids than astrocytes in response to CD14/TLR4 stimulation (Minghetti and Levi 1998). Microglia also respond to the prostanoids they secrete, expressing several prostaglandin receptors. Prostaglandin receptors are G-protein-coupled receptors classified by their relative ligand specificity for the different classes of prostanoids, but substantial cross-reactivity exists, and some receptors have equal affinity for multiple ligands. Microglia have been demonstrated to express the TP, EP2, and EP3 prostaglandin receptors that recognize thromboxane, PGE_2, and PGE_2/PGE_1, respectively (Kitanaka et al. 1996). The EP2 receptor, in particular, appears to play an important role in regulating microglia activation. Mice deficient in the EP2 receptor are protected from LPS-induced neurotoxicity, and EP2-deficient microglia fail to develop a neurotoxic phenotype following Aβ exposure, but nevertheless have enhanced Aβ phagocytic activity (Shie et al. 2005a,b). These reports suggest that specific antagonism of the microglia EP2 receptor might be a potential therapeutic target to lessen the impact of neuron inflammation in AD.

Microglia coordinate and direct CNS inflammation

While microglia are clearly capable of undergoing dramatic changes in function and gene expression after being activated, it is also becoming clear that the microglia cell may take the directorial role in coordinating the inflammatory response in the CNS (diagramed in Fig. 1). Following an activation signal, microglia cells can rapidly release intracellular mediators such as prostaglandins that are the products of enzymatic activity (Minghetti and Levi 1998) or cytokines stored in microvesicles (Bianco et al. 2005). These rapidly released mediators have multiple autocrine and paracrine effects. One key cast member in the inflammatory response that receives paracrine signals from microglia is the astrocyte. Astrocytes exposed to prostaglandin release glutamate and synergize with microglia to release TNFα, a neurotoxic cytokine (Bezzi et al. 2001). In addition, astrocytes that contribute to the blood–brain barrier may respond to inflammatory signals by weakening tight junctions (Duffy et al. 2000; Wachtel et al. 2001). This may result in the extravasation of inflammatory signaling molecules into the CNS capillary bed as well as the attraction of trafficking T lymphocytes or the migration of other leukocytes into the site of microglia activity. In the case of infection, microglia can recognize microbial products via the elaboration of surface Toll-like receptors and are induced to develop a phagocytic phenotype capable of presenting foreign antigens to invading T cells. Antigen-specific T cells will be stimulated to expand their population by the elaboration of proinflammatory cytokines from microglia.

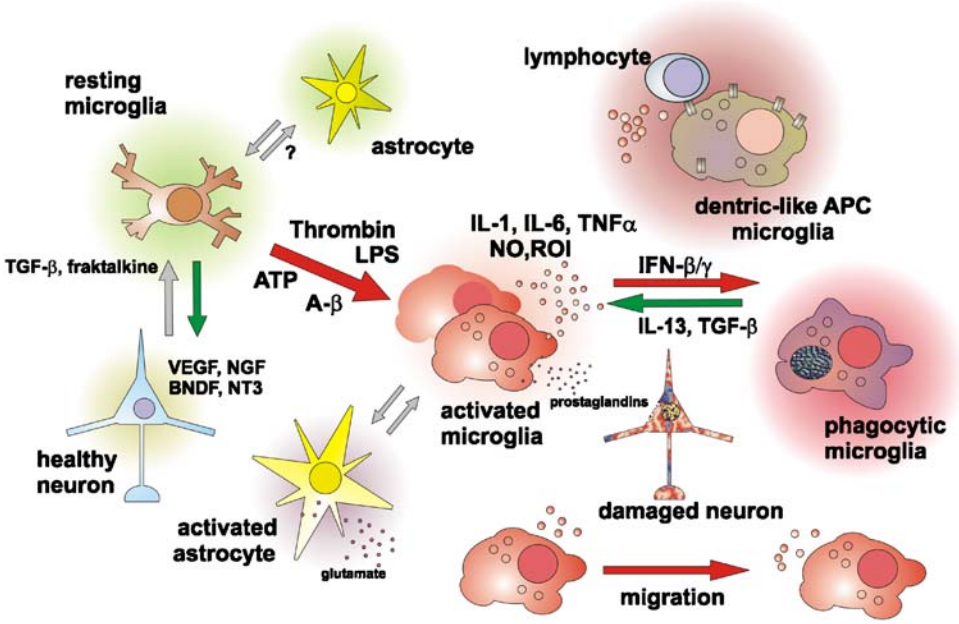

Fig. 1. A schematic diagram of microglia interactions in the CNS. Activated microglia can adopt a number of phenotypes including cytokine-secreting, phagocytic, and antigen-presenting cells. All of these different microglia phenotypes interact with each other as well as with astrocytes via the diffusible inflammatory mediators to produce a coordinated neuroinflammatory response.

Enlarging clones of T cells will establish a cell-mediated immune response to fight off pathogens, signal via the adaptive immune response for the generation of antibody, and/or contribute to the development of autoimmune CNS pathologies such as multiple sclerosis. When neuronal death occurs, microglia can recognize the elaboration of phosphatidylserine on the surface of apoptotic cells and will remove cellular debris from region by phagocytosis (Hirt et al. 2000; Witting et al. 2000; Hirt and Leist 2003). In addition, following stimulation with INF-β and INF-γ, microglia avidly phagocytose apoptotic T cells, which, in turn, serves as a signal to down-regulate the immune response (Chan et al. 2001, 2003; Magnus et al. 2001). Eventually, microglia write themselves out of the script (so to speak) by synthesis and release of IL-13 and TGFβ, anti-inflammatory cytokines that signal for microglia apoptosis (Yang et al. 2002). In an otherwise healthy CNS, when an injury or infection occurs over a discrete time frame, evidence for activated microglia in the region of injury eventually wanes, and remaining cells return to their ramified appearance. It is not currently known whether the microglia that remain dedifferentiate into a resting microglia, or if the activated microglia undergo apoptosis and the tissue is repopulated by resting microglia that migrate into a region of recovery or scar under a specific pattern of signals, perhaps elaborated by their dying brothers as they underwent cellular suicide. When this cycle of microglia activation and death fails to proceed as programmed, chronic neuroinflammation can ensue, either because the stimulus continues to be present (like Aβ) or because the immune response becomes dysregulated (such as in MS).

Microglia as a potential therapeutic target

Several means of modulating microglia responses are currently under investigation as potential future therapeutic interventions in a variety of neurodegenerative diseases as well as ischemic or traumatic CNS injury. Pharmacologic agents that prevent or prematurely down-regulate microglia responses to inflammatory signals have potential efficacy in CNS injury and disease in which inflammation is thought to elicit part of the observed neurotoxicity. One potentially useful agent with anti-inflammatory properties is minocycline, which likely has both antiapoptotic and anti-inflammatory mechanisms of action (Tikka and Koistinaho 2001; Wang et al. 2003b). Minocycline has demonstrated efficacy in animal models of CNS trauma, ischemia, and neurodegenerative diseases (Yrjanheikki et al. 1998; Wu et al. 2002; Hunter et al. 2004; Ryu et al. 2004; Stirling et al. 2004; Choi et al. 2005), and its efficacy in HD, PD, and ALS is currently under evaluation in clinical trials (Blum et al. 2004; Bonelli et al. 2004). However, the dominant mechanism by which minocycline protects CNS in the setting of disease or injury has not yet been determined. Traditional nonsteroidal anti-inflammatory agents, including selective and nonselective inhibitors of prostaglandin synthesis, have also been proposed as having potential therapeutic benefit in CNS injury and disease. Interestingly, recent work has shown that specific signaling through the EP2 prostaglandin receptor not only protects neurons from inflammatory injury induced by exposure to LPS or Aβ, but also promotes the microglia phagocytosis of fibrillar Aβ (Shie et al. 2005a,b). These studies suggest that the development of a pharmacological antagonist for the microglia EP2 receptor is a highly desirable therapeutic target. Biologically active peptides that modulate microglia function are also potential future molecular therapeutics for the treatment or prevention of CNS disease. For example, the tripeptide *Tuftsin fragment 1-3* (the peptide TKP) that inhibits microglia activation appears to provide protection against neuronal injury following axotomy (Thanos et al. 1993) and cerebral hemorrhage (Wang et al. 2003a). Finally, work by several groups has suggested that modulating the ability of the CNS immune system to respond either to acute or chronic injurious stimuli by immunologic methods may also be an important future therapeutic strategy (Benner et al. 2004; Buttini et al. 2005; Masliah et al. 2005). For example, both active and passive immunization approaches have generated promising results in animal models of AD (Gelinas et al. 2004), and active immunization against CNS antigens has provided protection from neuronal injury in animal models of CNS trauma and ischemia (Frenkel et al. 2003; Kipnis et al. 2003). Unfortunately, a human trial of active immunization against Aβ was halted early because of the development of diffuse autoimmune encephalitis (Gelinas et al. 2004), suggesting that the use of immunologic means to modulate CNS disease will require carefully nuanced approaches. Taken together, all of the above-mentioned means of modulating microglia function may be employed in the not too distant future toward the treatment or prevention of a multitude of CNS conditions for which there are currently few disease-modifying treatment approaches.

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