Microglia and Central Nervous System Immunity

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The central nervous system (CNS) has evolved as an immune-privileged site to protect its vital functions from damaging immune-mediated inflammation. With the protective shield of the blood-brain barrier and lack of lymphatic system, the CNS strictly regulates the entry of most peripheral immune cells, soluble factors, and plasma proteins in homeostasis.[1] However, during a CNS injury or disease such as multiple sclerosis (MS),[2] T-cell and peripheral antigen-presenting–cell (APC) infiltration are involved in the observed pathogenesis. Thus, there must be a CNS-adapted system of surveillance that continuously evaluates local changes in the nervous system and communicates to the peripheral immune system during an injury or a disease. Recent advances leading to a better understanding of the CNS disease processes has placed microglia, the CNS-based resident macrophages, at center stage in this system of active surveillance. Microglia are tightly regulated by their local environment, including neuron-microglia interactions during homeostasis, and they remain quiescent.[3] In the steady-state CNS, microglia cells express the macrophage marker CD11b and exhibit a resting phenotype characterized by a low-level expression of major histocompatibility complex (MHC) class I and II and co-stimulatory molecules like CD86 and CD40.[4–6] Conversely, in the event of an insult or injury, microglia cells increase mobility,[7] phagocytosis,[8,9] and proliferation,[10] and they contribute to resulting immune responses and inflammation by releasing various reactive oxygen intermediates, cytokines, and chemokines.[11] In comparison to macrophage responses in the peripheral tissues, microglia responses on activation are restricted, spatially and temporally, to preserve an immunologically silent CNS environment.[12] Microglia as APCs also contribute to activating T-cell responses by upregulating MHC and co-stimulatory molecules, generating specific CD4 and CD8 memory responses.[13–15] Therefore, microglia are essential components of the innate and adaptive aspects of immune responses in the CNS, similar to macrophages in the peripheral tissues.

Considering these broad immune functions, it is not surprising to see microglia accumulation in almost every CNS disease process; examples include CNS tumors, particularly malignant gliomas, which account for nearly half of primary CNS neoplasm and are known to accumulate a large number of microglia and a small population of lymphocytes. Initial observation of this high percentage of microglia in gliomas led many to deduce their role in antitumor activity and in cell death (necrosis) associated with many gliomas like glioblastoma multiforme (GBM). However, in recent years, the accumulating evidence strongly suggests that microglia cells contribute to the immunosuppressive environment of gliomas and can actually promote tumor growth.[16,17] This article discusses the role of microglia in CNS immunity and highlights key advances made in glioma immunology.

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ORIGIN OF MICROGLIA

Microglia constitute 5% to 20% of total glial cell population in the CNS, making them as abundant as neurons.[18–20] They were first recognized by Nissl, who named them ''Staebchenzellen'' or rod cells for their rod-shaped nuclei and considered them to be reactive neuroglia suggesting a capacity for migration and phagocytosis. In 1913, Santiago Ramon y Cajal described microglia as part of 3 elements of the CNS. It was del Rio Hortega, after his studies on young animals, who established microglia as distinct from other nonneuronal cell types, astrocytes and oligodendrocytes. Hortega described microglia's origin from mononuclear cells of the circulating blood and believed that these cells have the ability to transform from resting ramified form into amoeboid phagocytic macrophages.[21] These conclusions were based on Hortega's observations from stab wounds made in the mature brains of various animal species. In 1925, Wilder Penfield further usedthe special silver-staining method utilized by Hortega to provide the first detailed descriptions of microglia in glioma tissue. After a long debate over the role and origin of microglia in the CNS, they have been established as a phenotypically, developmentally, and functionally distinct population of glial cells that are of myelomonocytic origin. The microglia precursor cells of monocyte-macrophage lineage are derived from mesodermal hematopoietic cells, which in mammals originate from the yolk sac.[22] These circulating precursor cells invade the developing brain during perinatal stages and transform into microglia cells that express several macrophage-specific markers, including Toll-like receptors (TLRs),[23,24] the integrin CD11b,[25] and the glycoprotein of unknown function, F4/80, but they have lower levels of the leukocyte common antigen, CD45, when compared with macrophages.[26,27] These findings together with the phagocytic activity of microglia strongly suggest that microglia are closely related to peripheral monocytes.

Recent studies have classified microglia into 3 types according to their morphologic appearance: resting ramified microglia, activated reactive microglia, and amoeboid phagocytic microglia.[22] Furthermore, consistent with the immunologically silent nature of the CNS, the invading amoeboid microglia in perinatal life transform into ramified resting microglia during postnatal life, which represent a fairly permanent population with slower turnover when compared with macrophages of other tissues.[28,29] According to studies done by Lawson and colleagues,[19,20] the resting ramified microglia are less numerous in white matter than in gray matter, and they adapt the morphology of their cell bodies, processes, and expression of cell surface markers to their microenvironment. The microglia remain in the resting state until a stimulus from an injury, infection, or other neurodegenerative process activates them to transform back into activated amoeboid phagocytic cells.[8,9]

MICROGLIA AS A SYSTEM OF ACTIVE SURVEILLANCE

In the steady state, the fairly quiescent-looking microglia cells undergo continuous pinocytosis to sample the surrounding microenvironment, as a way of conducting routine surveillance of the CNS.[30] Neuron-to-microglia communication also plays a key role in the surveillance, and it shapes the quiescent and reactive states of microglia. In vitro studies have provided evidence that electrically active neurons inhibit T_H1 cytokine interferon (IFN)-γ-induced expression of major histocompatibility complex (MHC) class II molecules on astrocytes and microglia,[31] whereas some neurotransmitter molecules like substance P[32] enhance the pro-inflammatory phenotype of microglia. These opposing actions of various neurotransmitters suggest the existence of a complex set of interactions between local inhibitory and stimulatory signals in shaping microglia responses. In vivo studies conducted by Hoek and colleagues[33,34] have led to the characterization of a previously identified membrane-bound glycoprotein termed OX2 or CD200 expressed on neurons as a key regulator of microglia. In OX2-deficient mice, microglia exhibit an activated phenotype with enhanced CD45 and complement type-3 receptor (CR3) along with less ramified morphology. In addition, OX2- deficient mice show an accelerated reactive response to an injury. Thus, neurons deliver inhibitory signals to microglia through OX2 receptor signaling pathway. These findings in combination strongly support the idea of microenvironment regulation of microglia function.

Moreover, the plasticity of microglia in function and morphology directly correlates with its interactions with the microenvironment. Granulocyte-macrophage colony-stimulating factor (GM-CSF) and M-CSF are known to play a crucial role in the terminal differentiation of tissue macrophages.[35,36] In Alzheimer disease and MS, it has been shown that levels of GM-CSF are elevated in addition to upregulation in M-CSF receptor.[37,38] With in vitro studies, Fischer and Reichmann have demonstrated that the incubation of purified microglia with GM-CSF increases microglia cell size, and it generates a heterogeneous population that contains cells resembling other tissue macrophages and microglia cells expressing CD11c and MHC class II molecules.[39] These studies indicate that microglia cells are poised to use their range of plasticity in response to their microenvironment changes.

MICROGLIA AS MEDIATORS OF INFLAMMATION

One key similarity of microglia and peripheral macrophages is the ability to contribute significantly to innate and adaptive immune responses. The resting microglia cells are activated by various stimuli, such as lipopolysaccharide (LPS), β amyloid, IFN-γ, thrombin, and some proinflammatory cytokines, involved in infection, neurodegenerative diseases, and CNS injury.[40] Upon stimulation, the resting ramified microglia undergo a series of morphologic and functional changes to mobilize the cellular and molecular defense of the CNS. For example, it has been demonstrated that microglia express TLRs[23] that interact with bacterial cell wall components to initiate innate response such as production of cytokines, chemokines, and nitric oxide.[41,42] The molecules released by microglia in response to stimuli include (1) proinflammatory cytokines interleukin (IL)-1,[43] IL-6,[44] and tumor necrosis factor (TNF)-α[45]; and (2) monocyte chemoattractant protein-1 (MCP-1),[46] macrophage inflammatory protein 1 (MIP-1),[47,48] and regulated on activation normal T cell expressed and secreted (RANTES)[49]; and (3) chemokines involved in lymphocyte recruitment. This implicates microglia as a critical first line of defense because the adaptive immune cells typically take longer to respond to pathogens present in the CNS. Once the microenvironment of the CNS has become activated, the local cells produce proinflammatory cytokines and chemokines and upregulate immunomodulatory surface markers to contribute to local inflammatory response in addition to decreasing the stringency of the blood brain barrier. This allows the entry of soluble factors and immune effector cells from the periphery,[50] such as macrophages, natural killer cells, and lymphocytes.[51,52] Early activation of microglia before peripheral cell infiltration is supported by bone marrow chimera studies demonstrating that microglia activation precedes the entry of peripheral macrophages.[35] Microglia also exhibit phagocytic and cytotoxic functions during CNS infection and injury. On microglia activation, there is upregulation of opsonic receptors, including complement receptors (CR1, CR3, CR4) and FcγR (I,II,III), which enhances phagocytosis by binding to complement components and immunoglobulin fragments respectively.[53,54] Microglia are known to function as transitory phagocytes during CNS ontogeny to clear apoptotic neuronal cell bodies,[55] and in experimental autoimmune encephalitis (EAE), the animal model of MS, T-cell debris is phagocytosed by microglia.[56] Phagocytosis by microglia directly induces the production of reactive radicals to degrade cellular debris. Moreover, microglia secrete superoxide radicals and nitric oxide into their microenvironment in response to pathogens and cytokine stimulation.[57]

Antigen presentation is a critical event involved in the generation of T-cell responses against the pathogen, as part of pathogen-specific adaptive immune response. The antigen presentation requires interaction between the T-cell receptor and the processed antigen peptide presented on MHC molecules on the surface of APCs. MHC class I and MHC class II molecules stimulate CD8 cytotoxic cells and CD4 T-helper cells, respectively. Additional key interactions between co-stimulatory molecules, such as B7-1 (CD80), B7-2 (CD86), and CD40, expressed on the surface of APC and specific counter-receptors on T cells are required for optimal T-cell–APC adhesion and activation.[58,59] Within the CNS parenchyma under steady-state conditions, MHC class I and II expression is generally minimal or absent, and when present, it is restricted to microglia in low levels.[6,60,61] According to the studies done by Ford and colleagues[62] in normal rodent brains, microglia behave as poor APCs under resting conditions. However, in inflammatory and neurodegenerative conditions, microglia readily upregulate MHC expression[63] along with co-stimulatory molecules.[64] In particular, the interaction of CD80 and CD86 molecules on microglia with CD28 expressed on T cells is required for inducing T-cell cytokine secretion, growth, and survival.[58] Additionally, microglia CD40 interaction with CD40L on T cells enhances expression of MHC class II, nitric synthase and CD80/CD86 molecules on microglia, which in turn further promote T-cell activation.[59] In EAE, the animal model of MS, both microglia and blood-derived macrophages have been shown to express MHC class II and co-stimulatory molecules.[65] Additionally, in vivo and in vitro studies have demonstrated that IFN-γ, a cytokine secreted by pre-activated CD4 T cells and NK cells, induces and maintains MHC class II and adhesion/co-stimulatory molecule expression on microglia to maintain stimulation of T cells.[66]

In addition to initiating innate and adaptive immune responses, microglia contribute to downregulating inflammatory responses. In the absence of sufficient co-stimulatory molecules, the interaction of Fas ligand (FasL) on APCs with the Fas receptor on the T cell will lead to activation-induced T cell apoptosis.[67] In vitro[68,69] and in vivo studies of EAE[70] have detected FasL expression on microglia. Cytotoxic molecules like nitric oxide produced by microglia can also contribute to the death of immune effector response. Additionally, microglia can express Fas molecules on their surface that can interact with FasL-expressing cells, leading to their own apoptosis.[70] These studies indicate that microglia activity in a neuropathology can be self-limiting in addition to regulating other immune effector cells.

MICROGLIA IN CNS GLIOMAS

Microglia Chemoattraction and Proliferation in Glioma

Histopathologic studies of glioma tissue have consistently identified a high infiltrating population of microglia within gliomas.[71–75] Badie and colleagues[75] using flow cytometry showed one-third of glioma cells to be expressing resident microglia markers, and this exceeds the number of other immune cells in gliomas. It has been proposed that microglia accumulation at the site of a CNS tumor is due to the local production of chemoattractants and growth factors. For example, MCP-1 is produced by glioma cells, and microglia have been found to express the MCP-1 receptor, CCR2, on their surface.[76,77] Thus, local production of MCP-1 may help recruit microglia to the site of glioma. Furthermore, growth factors such as colony stimulating factor-1 (CSF-1), granulocyte colony-stimulating factor (G-CSF) and hepatocyte growth factor/scatter factor (HGF/SF) have been known to be secreted by various gliomas,[78–80] and these growth factors along with serving chemokine signals also promote proliferation of microglia. Therefore, gliomas are actively promoting microglia trafficking and proliferation by secreting chemokines and growth factors.

Microglia's Role in Tumor Progression

The recruitment of microglia in brain tumors was postulated as an attempt by the CNS to destroy invading neoplastic cells.[81] However, in recent years, it has become increasingly evident that the immune defense functions of microglia against glioma are compromised because there is no strong evidence of tumor rejection, and tumors grow despite increased microglia invasion over time. Also, the microglia, instead of being localized to the area of necrosis, are found diffusely throughout tumor parenchyma and do not appear to be phagocytosing tumor cells or debris.[2] Growing evidence suggests that glioma infiltrating microglia/macrophages may actually be promoting tumor growth.[82] For instance, though MHC molecule expression has been detected on microglia cells in gliomas,[83,84] they appear to be deficient in proper antigen presentation[85] for cytotoxic and helper T-cell activation. The number of microglia cells expressing MHC class II antigen is further reduced in high-grade gliomas, despite the abundance of microglia in these CNS neoplasms. Schartner and colleagues[16] demonstrated that microglia isolated from tumor-expressing mice do not show MHC class II upregulation following in vivo stimulation, thereby preventing the induction of an efficient antitumor immune response. Additionally, the expression of MHC class II and co-stimulatory B7 molecules was significantly increased when freshly isolated microglia were cultured in the absence of glioma cells.[86] Alternatively, the interaction of microglia with T cells can inhibit T-cell function by increased expression of inhibitory molecules. In particular, increased expression of co-stimulatory molecule B7-H1 on APCs induces T cell apoptosis. Microglia express B7-H1 in homeostasis, but the expression is upregulated in pathologic conditions including gliomas.[87,88] Thus, it is likely that upregulation of inhibitory molecules like B7-H1 may contribute to inhibition of T-cell activation, and even apoptosis, regardless of the presence of MHC molecules.[89]

FasL is another inhibitory molecule expressed on tumor-associated microglia that may play a key role in limiting the ability of T cells to recognize and respond to tumor cells. FasL-expressing cells induce apoptosis of CD8 T cells and thus, the upregulation of FasL on tumor-associated microglia may serve as another mechanism to limit tumor recognition by cytotoxic cells. Microglia in intracranial tumors express FasL and the inhibition of FasL activity has been demonstrated to dramatically increase the number of peripheral immune effector cells in tumors.[90] In a study by Parney and colleagues,[91] coculturing human peripheral blood mononuclear cells with human glioma lines caused glioma-conditioned monocytes to reduce phagocytosis and induce apoptosis of activated lymphocytes when added to the glioma-monocyte culture. Kostianovsky and colleagues[92] studied microglia/monocyte activation in GBM. They found that the presence of GBM tumor cells down-regulated the production of the proinflammatory cytokine TNF-α by microglia after LPS stimulation and instead, anti-inflammatory cytokine IL-10 was induced. The immunosuppression by microglia was maintained with different stimulus signals such as β amyloid, suggesting that GBM tumor cells affect microglia/monocyte activation regardless of the nature of stimuli. Moreover, glioma cells produce anti-inflammatory cytokines, such as IL-6, transforming growth factor (TGF)-β2, and prostaglandin E2, and tumor-growth–promoting cytokines, such as IL-1 and bFGF.[2,93] TGF-β2 in particular inhibits proliferation and secretion of pro-inflammatory cytokines by microglia and lymphocytes.[94] These studies together suggest that the phagocytosis, pro-inflammatory cytokine secretion, and antigen presentation functions of microglia are strongly suppressed by glioma cells.

Microglia, under the influence of an immunosuppressive environment, also secrete anti-inflammatory cytokines such as IL-10 that enable rapid proliferation of tumor cells by inhibiting cytotoxic T cell.[95] Additionally in vitro studies have demonstrated that IL-10 suppresses microglia antigen presentation function by inhibiting IFN-γ–induced MHC class II expression.[96] Thus, microglia IL-10 secretion further contributes to the maintenance of an immunosuppressive environment. Microglia are known to be the cellular source of matrix metalloproteinase-2 (MMP-2), extracellular matrix-degrading enzymes, and their release into the tumor environment can help increase the spread of tumors because in the absence of extracellular matrix, tumor cells are free to proliferate.[97] Microglia also secret tumor proliferation promoting factors including epidermal growth factor (EGF) and vascular endothelial growth factor (VEGF),[38,98] which are well known to be involved in glial cell proliferation and tumor angiogenesis. These studies in combination suggest that microglia play an integrative role in the tumor progression by supporting migration (MMP-9), angiogenesis (VEGF), and proliferation (EGF) of glioma cells.

Therapeutic Potential of Microglia in Glioma

Microglia are potent immune effector cells that display a broad range of functionality, mediating both innate and adaptive responses during CNS injury and disease while remaining quiescent in the steady state. In addition to their significant numbers in gliomas, their versatility in bridging the gap between the immune-privileged CNS and the peripheral immune system makes them a potential candidate in immunotherapy treatment. Microglia associated with CNS tumors do not seem to be active in inducing antitumor T-cell response; there is potential for overcoming glioma-induced immunosuppression, so that activated microglia can enhance CNS immunity against tumors. For instance, signal transducer and activator of transcription (Stat) 3 protein inhibitors have been found to activate microglia and T cell immune responses in malignant glioma.[92,99–101] Signal transducers and activators of transcription proteins are a family of transcription factors activated by tyrosine kinases such as epidermal growth factor receptor (EGFR) in response to tumor-secreted factors including VEGF, IL-6, and IL-10.[102,103] On activation, the Stat proteins translocate to the nucleus and bind to specific elements within target promoters to regulate gene expression.[104] Malignant gliomas are well known for their dysregulation of the EGFR pathway via amplification, mutation of EGFR, and upregulation of Stat3 activation.[105–108] Stat signaling, in particular Stat3, regulates immune activation and tolerance by altering transcription of several genes that control tumor cell survival, resistance to apoptosis, cell cycle progression, and angiogenesis in glioma cells.[109] These findings suggest that Stat3 is abnormally activated through EGFR dysregulation and contributes to glioma proliferation and immunosuppression.

In vitro studies, Iwamaru and colleagues[100] demonstrated that a novel small molecule, WP1066—a pharmacologic derivative of the natural compound caffeic acid—inhibits Stat3 activation. Stat3 signaling inhibition downregulates gene expression of Bcl-XL, Mcl-1, and c-myc and induces apoptosis resulting in significant inhibition of tumor cell growth in human malignant glioma cell lines U87-MG and U373-MG. Studies by Hussain and colleagues[99] demonstrated that Stat3 inhibition by WP1066 reversed immune tolerance in GBM by upregulating co-stimulatory molecules CD80 and CD86 in glioma-infiltrating microglia and peripheral blood monocytes and inducing effector T cell–stimulating cytokines, such as IL-2, IL-4,IL-12, and IL-15. Collectively, these studies indicate that the inhibition of Stat3 activity could be a promising therapeutic strategy for the treatment of patients with malignant gliomas due to its potent immune adjuvant responses.

Additionally, studies by Carpentier and colleagues[110,111] demonstrated long-term survival in animals with established CNS glioma using single intratumoral injection of CpG oligodeoxynucleotide, an immunostimulatory sequence that signals through TLR9 to induce production of IFN-α, IFN-β, IL-12, and TNF-α. However, animals with macrophage/microglia depletion were unable to reject tumor after CpG treatment, showing that microglia/macrophages are a critical component of antitumor response.[112] Though CpG is fairly safe in humans, an improved understanding of microglia function in gliomas can provide better methods of manipulating the glioma microenvironment to allow effective antigen presentation to T cells.

REFERENCES

1. Pachter JS, de Vries HE, Fabry Z. The blood-brain barrier and its role in immune privilege in the central nervous system. J Neuropathol Exp Neurol 2003;62(6):593–604.
2. Hao C, Parney IF, Rao WH, et al. Cytokine and cytokine receptor mRNA expression in human glioblastomas: evidence of Th1, Th2 and Th3 cytokine dysregulation. Acta Neuropathol (Berl) 2002; 103(2):171–8.
3. Ayoub AE, Salm AK. Increased morphological diversity of microglia in the activated hypothalamic supraoptic nucleus. J Neurosci 2003;23(21):7759–66.
4. Sedgwick JD, Schwender S, Imrich H, et al. Isolation and direct characterization of resident microglial cells from the normal and inflamed central nervous system. Proc Natl Acad Sci U S A 1991; 88(16):7438–42.
5. Aloisi F, De Simone R, Columba-Cabezas S, et al. Functional maturation of adult mouse resting microglia into an APC is promoted by granulocyte-macrophage colony-stimulating factor and interaction with Th1 cells. J Immunol 2000;164(4): 1705–12.
6. Hoftberger R, Aboul-Enein F, Brueck W, et al. Expression of major histocompatibility complex class I molecules on the different cell types in multiple sclerosis lesions. Brain Pathol 2004;14(1): 43–50.
7. Carbonell WS, Murase SI, Horwitz AF, et al. Infiltrative microgliosis: activation and long-distance migration of subependymal microglia following periventricular insults. J Neuroinflammation 2005; 2(1):5.
8. Schroeter M, Jander S, Huitinga I, et al. Phagocytic response in photochemically induced infarction of rat cerebral cortex. The role of resident microglia. Stroke 1997;28(2):382–6.
9. Zhang SC, Goetz BD, Carre JL, et al. Reactive microglia in dysmyelination and demyelination. Glia 2001;34(2):101–9.
10. Eliason DA, Cohen SA, Baratta J, et al. Local proliferation of microglia cells in response to neocortical injury in vitro. Brain Res Dev Brain Res 2002;137(1): 75–9.
11. Liu L, Persson JK, Svensson M, et al. Glial cell responses, complement, and clusterin in the central nervous system following dorsal root transection. Glia 1998;23(3):221–38.
12. Aloisi F. Immune function of microglia. Glia 2001; 36(2):165–79.
13. Brannan CA, Roberts MR. Resident microglia from adult mice are refractory to nitric oxide-inducing stimuli due to impaired NOS2 gene expression. Glia 2004;48(2):120–31.
14. Aloisi F, Ria F, Penna G, et al. Microglia are more efficient than astrocytes in antigen processing and in Th1 but not Th2 cell activation. J Immunol 1998;160(10):4671–80.
15. Aloisi F, Ria F, Columba-Cabezas S, et al. Relative efficiency of microglia, astrocytes, dendritic cells and B cells in naive CD4+ T cell priming and Th1/Th2 cell restimulation. Eur J Immunol 1999; 29(9):2705–14.
16. Schartner JM, Hagar AR, Van Handel M, et al. Impaired capacity for upregulation of MHC class II in tumor-associated microglia. Glia 2005;51(4):279–85.
17. Bettinger I, Thanos S, Paulus W. Microglia promote glioma migration. Acta Neuropathol 2002;103(4): 351–5.
18. Benveniste EN. Role of macrophages/microglia in multiple sclerosis and experimental allergic encephalomyelitis. J Mol Med 1997;75(3):165–73.
19. Lawson LJ, Perry VH, Dri P, et al. Heterogeneity in the distribution and morphology of microglia in the normal adult mouse brain. Neuroscience 1990;39(1):151–70.
20. Lawson LJ, Perry VH, Gordon S. Turnover of resident microglia in the normal adult mouse brain. Neuroscience 1992;48(2):405–15.
21. Del Rio-Hortega P. Microglia. In: Penfield W, editor. Cytology and cellular pathology of the nervous system, vol. 11. New York: Paul B Hoeber; 1932. p. 481–534.
22. Ling EA, Wong WC. The origin and nature of ramified and amoeboid microglia: a historical review and current concepts. Glia 1993;7(1):9–18.
23. Bsibsi M, Ravid R, Gveric D, et al. Broad expression of Toll-like receptors in the human central nervous system. J Neuropathol Exp Neurol 2002; 61(11):1013–21.
24. Olson JK, Miller SD. Microglia initiate central nervous system innate and adaptive immune responses through multiple TLRs. J Immunol 2004;173(6):3916–24.
25. Akiyama H, McGeer PL. Brain microglia constitutively express beta-2 integrins. J Neuroimmunol 1990;30(1):81–93.
26. Dick AD, Ford AL, Forrester JV, et al. Flow cytometric identification of a minority population of MHC class II positive cells in the normal rat retina distinct from CD45lowCD11b/c+CD4low parenchymal microglia. Br J Ophthalmol 1995;79(9): 834–40.
27. Becher B, Antel JP. Comparison of phenotypic and functional properties of immediately ex vivo and cultured human adult microglia. Glia 1996;18(1):1–10.
28. Hess DC, Abe T, Hill WD, et al. Hematopoietic origin of microglial and perivascular cells in brain. Exp Neurol 2004;186(2):134–44.

29. Kennedy DW, Abkowitz JL. Kinetics of central nervous system microglial and macrophage engraftment: analysis using a transgenic bone marrow transplantation model. Blood 1997;90(3):986–93.
30. Nimmerjahn A, Kirchhoff F, Helmchen F. Resting microglial cells are highly dynamic surveillants of brain parenchyma in vivo. Science 2005; 308(5726):1314–8.
31. Neumann H, Boucraut J, Hahnel C, et al. Neuronal control of MHC class II inducibility in rat astrocytes and microglia. Eur J Neurosci 1996;8(12):2582–90.
32. McCluskey LP, Lampson LA. Local neurochemicals and site-specific immune regulation in the CNS. J Neuropathol Exp Neurol 2000;59(3):177–87.
33. Hoek RM, Ruuls SR, Murphy CA, et al. Down-regulation of the macrophage lineage through interaction with OX2 (CD200). Science 2000;290(5497): 1768–71.
34. Wright GJ, Puklavec MJ, Willis AC, et al. Lymphoid/neuronal cell surface OX2 glycoprotein recognizes a novel receptor on macrophages implicated in the control of their function. Immunity 2000;13(2): 233–42.
35. Schilling M, Besselmann M, Leonhard C, et al. Microglial activation precedes and predominates over macrophage infiltration in transient focal cerebral ischemia: a study in green fluorescent protein transgenic bone marrow chimeric mice. Exp Neurol 2003;183(1):25–33.
36. De Simone R, Ajmone-Cat MA, Tirassa P, et al. Apoptotic PC12 cells exposing phosphatidylserine promote the production of anti-inflammatory and neuroprotective molecules by microglial cells. J Neuropathol Exp Neurol 2003;62(2):208–16.
37. Kreuzfelder E, Menne S, Ferencik S, et al. Assessment of peripheral blood mononuclear cell proliferation by [2-3H]adenine uptake in the woodchuck model. Clin Immunol Immunopathol 1996;78(3): 223–7.
38. Tsai JC, Goldman CK, Gillespie GY. Vascular endothelial growth factor in human glioma cell lines: induced secretion by EGF, PDGF-BB, and bFGF. J Neurosurg 1995;82(5):864–73.
39. Fischer HG, Reichmann G. Brain dendritic cells and macrophages/microglia in central nervous system inflammation. J Immunol 2001;166(4): 2717–26.
40. Dheen ST, Kaur C, Ling EA. Microglial activation and its implications in the brain diseases. Curr Med Chem 2007;14(11):1189–97.
41. Banati RB, Gehrmann J, Schubert P, et al. Cytotoxicity of microglia. Glia 1993;7(1):111–8.
42. Nakamichi K, Saiki M, Sawada M, et al. Double-stranded RNA stimulates chemokine expression in microglia through vacuolar pH-dependent activation of intracellular signaling pathways. J Neurochem 2005;95(1):273–83.
43. Hartlage-Rubsamen M, Lemke R, Schliebs R. Interleukin-1beta, inducible nitric oxide synthase, and nuclear factor-kappaB are induced in morphologically distinct microglia after rat hippocampal lipopolysaccharide/interferon-gamma injection. J Neurosci Res 1999;57(3):388–98.
44. Suzumura A, Sawada M, Marunouchi T. Selective induction of interleukin-6 in mouse microglia by granulocyte-macrophage colony-stimulating factor. Brain Res 1996;713(1–2):192–8.
45. Floden AM, Li S, Combs CK. Beta-amyloid-stimulated microglia induce neuron death via synergistic stimulation of tumor necrosis factor alpha and NMDA receptors. J Neurosci 2005;25(10):2566–75.
46. Babcock AA, Kuziel WA, Rivest S, et al. Chemokine expression by glial cells directs leukocytes to sites of axonal injury in the CNS. J Neurosci 2003;23(21): 7922–30.
47. Si Q, Cosenza M, Zhao ML, et al. GM-CSF and M-CSF modulate beta-chemokine and HIV-1 expression in microglia. Glia 2002;39(2):174–83.
48. Takami S, Nishikawa H, Minami M, et al. Induction of macrophage inflammatory protein MIP-1alpha mRNA on glial cells after focal cerebral ischemia in the rat. Neurosci Lett 1997;227(3):173–6.
49. Chen CJ, Chen JH, Chen SY, et al. Upregulation of RANTES gene expression in neuroglia by Japanese encephalitis virus infection. J Virol 2004; 78(22):12107–19.
50. Lu J, Moochhala S, Kaur C, et al. Cellular inflammatory response associated with breakdown of the blood-brain barrier after closed head injury in rats. J Neurotrauma 2001;18(4):399–408.
51. Tonra JR, Reiseter BS, Kolbeck R, et al. Comparison of the timing of acute blood-brain barrier breakdown to rabbit immunoglobulin G in the cerebellum and spinal cord of mice with experimental autoimmune encephalomyelitis. J Comp Neurol 2001;430(1):131–44.
52. Baldwin AC, Kielian T. Persistent immune activation associated with a mouse model of Staphylococcus aureus-induced experimental brain abscess. J Neuroimmunol 2004;151(1–2):24–32.
53. Peress NS, Fleit HB, Perillo E, et al. Identification of Fc gamma RI, II and III on normal human brain ramified microglia and on microglia in senile plaques in Alzheimer's disease. J Neuroimmunol 1993;48(1):71–9.
54. Barnum SR. Inhibition of complement as a therapeutic approach in inflammatory central nervous system (CNS) disease. Mol Med 1999;5(9):569–82.
55. Ferrer I, Bernet E, Soriano E, et al. Naturally occurring cell death in the cerebral cortex of the rat and removal of dead cells by transitory phagocytes. Neuroscience 1990;39(2):451–8.
56. Nguyen KB, Pender MP. Phagocytosis of apoptotic lymphocytes by oligodendrocytes in experimental

autoimmune encephalomyelitis. Acta Neuropathol 1998;95(1):40–6.

57. Chao CC, Hu S, Peterson PK. Modulation of human microglial cell superoxide production by cytokines. J Leukoc Biol 1995;58(1):65–70.
58. Slavik JM, Hutchcroft JE, Bierer BE. CD28/CTLA-4 and CD80/CD86 families: signaling and function. Immunol Res 1999;19(1):1–24.
59. van Kooten C, Banchereau J. CD40-CD40 ligand. J Leukoc Biol 2000;67(1):2–17.
60. Stoll M, Capper D, Dietz K, et al. Differential microglial regulation in the human spinal cord under normal and pathological conditions. Neuropathol Appl Neurobiol 2006;32(6):650–61.
61. Gehrmann J, Matsumoto Y, Kreutzberg GW. Microglia: intrinsic immuneffector cell of the brain. Brain Res Brain Res Rev 1995;20(3):269–87.
62. Ford AL, Foulcher E, Lemckert FA, et al. Microglia induce CD4 T lymphocyte final effector function and death. J Exp Med 1996;184(5):1737–45.
63. Kreutzberg GW. Microglia: a sensor for pathological events in the CNS. Trends Neurosci 1996; 19(8):312–8.
64. De Simone R, Giampaolo A, Giometto B, et al. The costimulatory molecule B7 is expressed on human microglia in culture and in multiple sclerosis acute lesions. J Neuropathol Exp Neurol 1995;54(2): 175–87.
65. Li H, Cuzner ML, Newcombe J. Microglia-derived macrophages in early multiple sclerosis plaques. Neuropathol Appl Neurobiol 1996;22(3):207–15.
66. Shrikant P, Benveniste EN. The central nervous system as an immunocompetent organ: role of glial cells in antigen presentation. J Immunol 1996; 157(5):1819–22.
67. Pender MP, Rist MJ. Apoptosis of inflammatory cells in immune control of the nervous system: role of glia. Glia 2001;36(2):137–44.
68. Spanaus KS, Schlapbach R, Fontana A. TNF-alpha and IFN-gamma render microglia sensitive to Fas ligand-induced apoptosis by induction of Fas expression and down-regulation of Bcl-2 and Bcl-xL. Eur J Immunol 1998;28(12):4398–408.
69. Frigerio S, et al. Modulation of fas-ligand (Fas-L) on human microglial cells: an in vitro study. J Neuroimmunol 2000;105(2):109–14.
70. Kohji T, Matsumoto Y. Coexpression of Fas/FasL and Bax on brain and infiltrating T cells in the central nervous system is closely associated with apoptotic cell death during autoimmune encephalomyelitis. J Neuroimmunol 2000;106(1–2):165–71.
71. Rossi ML, Jones NR, Candy E, et al. The mononuclear cell infiltrate compared with survival in high-grade astrocytomas. Acta Neuropathol 1989; 78(2):189–93.
72. Roggendorf W, Strupp S, Paulus W. Distribution and characterization of microglia/macrophages in human brain tumors. Acta Neuropathol 1996; 92(3):288–93.
73. Streit WJ. Cellular immune response in brain tumors. Neuropathol Appl Neurobiol 1994;20(2): 205–6.
74. Wierzba-Bobrowicz T, Kuchna I, Matyja E. Reaction of microglial cells in human astrocytomas (preliminary report). Folia Neuropathol 1994;32(4):251–2.
75. Badie B, Schartner JM, Paul J, et al. Dexamethasone-induced abolition of the inflammatory response in an experimental glioma model: a flow cytometry study. J Neurosurg 2000;93(4):634–9.
76. Prat E, Baron P, Meda L, et al. The human astrocytoma cell line U373MG produces monocyte chemotactic protein (MCP)-1 upon stimulation with beta-amyloid protein. Neurosci Lett 2000; 283(3):177–80.
77. Galasso JM, Stegman LD, Blaivas M, et al. Experimental gliosarcoma induces chemokine receptor expression in rat brain. Exp Neurol 2000;161(1): 85–95.
78. Suzuki Y, Funakoshi H, Machide M, et al. Regulation of cell migration and cytokine production by HGF-like protein (HLP)/macrophage stimulating protein (MSP) in primary microglia. Biomed Res 2008;29(2):77–84.
79. Alterman RL, Stanley ER. Colony stimulating factor-1 expression in human glioma. Mol Chem Neuropathol 1994;21(2-3):177–88.
80. Badie B, Schartner J, Klaver J, et al. In vitro modulation of microglia motility by glioma cells is mediated by hepatocyte growth factor/scatter factor. Neurosurgery 1999;44(5):1077–82 [discussion: 1082–3].
81. Morantz RA, Wood GW, Foster M, et al. Macrophages in experimental and human brain tumors. Part 2: studies of the macrophage content of human brain tumors. J Neurosurg 1979;50(3): 305–11.
82. Badie B, Schartner J. Role of microglia in glioma biology. Microsc Res Tech 2001;54(2):106–13.
83. Proescholdt MA, Merrill MJ, Ikejiri B, et al. Site-specific immune response to implanted gliomas. J Neurosurg 2001;95(6):1012–9.
84. Tran CT, Wolz P, Egensperger R, et al. Differential expression of MHC class II molecules by microglia and neoplastic astroglia: relevance for the escape of astrocytoma cells from immune surveillance. Neuropathol Appl Neurobiol 1998;24(4):293–301.
85. Flugel A, Labeur MS, Grasbon-Frodl EM, et al. Microglia only weakly present glioma antigen to cytotoxic T cells. Int J Dev Neurosci 1999;17(5–6): 547–56.
86. Badie B, Bartley B, Schartner J. Differential expression of MHC class II and B7 costimulatory molecules by microglia in rodent gliomas. J Neuroimmunol 2002;133(1–2):39–45.

87. Magnus T, Schreiner B, Korn T, et al. Microglial expression of the B7 family member B7 homolog 1 confers strong immune inhibition: implications for immune responses and autoimmunity in the CNS. J Neurosci 2005;25(10):2537–46.
88. Parsa AT, Waldron JS, Panner A, et al. Loss of tumor suppressor PTEN function increases B7-H1 expression and immunoresistance in glioma. Nat Med 2007;13(1):84–8.
89. Dong H, Strome SE, Salomao DR, et al. Tumor-associated B7-H1 promotes T-cell apoptosis: a potential mechanism of immune evasion. Nat Med 2002;8(8):793–800.
90. Badie B, Schartner J, Prabakaran S, et al. Expression of Fas ligand by microglia: possible role in glioma immune evasion. J Neuroimmunol 2001; 120(1–2):19–24.
91. Parney IF, Waldron JS, Parsa AT. Flow cytometry and in vitro analysis of human glioma-associated macrophages. J Neurosurg 2009;110(3):572–82.
92. Kostianovsky AM, Maier LM, Anderson RC, et al. Astrocytic regulation of human monocytic/microglial activation. J Immunol 2008;181(8):5425–32.
93. Parney IF, Hao C, Petruk KC. Glioma immunology and immunotherapy. Neurosurgery 2000;46(4): 778–91 [discussion: 791–2].
94. Suzumura A, Sawada M, Yamamoto H, et al. Transforming growth factor-beta suppresses activation and proliferation of microglia in vitro. J Immunol 1993;151(4):2150–8.
95. Hishii M, Nitta T, Ishida H, et al. Human glioma-derived interleukin-10 inhibits antitumor immune responses in vitro. Neurosurgery 1995;37(6):1160–6 [discussion: 1166–7].
96. O'Keefe GM, Nguyen VT, Benveniste EN. Class II transactivator and class II MHC gene expression in microglia: modulation by the cytokines TGF-beta, IL-4, IL-13 and IL-10. Eur J Immunol 1999;29(4): 1275–85.
97. Rao JS. Molecular mechanisms of glioma invasiveness: the role of proteases. Nat Rev Cancer 2003; 3(7):489–501.
98. Lafuente JV, Adan B, Alkiza K, et al. Expression of vascular endothelial growth factor (VEGF) and platelet-derived growth factor receptor-beta (PDGFR-beta) in human gliomas. J Mol Neurosci 1999;13(1–2):177–85.
99. Hussain SF, Kong LY, Jordan J, et al. A novel small molecule inhibitor of signal transducers and activators of transcription 3 reverses immune tolerance in malignant glioma patients. Cancer Res 2007; 67(20):9630–6.
100. Iwamaru A, Szymanski S, Iwado E, et al. A novel inhibitor of the STAT3 pathway induces apoptosis in malignant glioma cells both in vitro and in vivo. Oncogene 2007;26(17):2435–44.
101. Zhang L, Alizadeh D, Van Handel M, et al. Stat3 inhibition activates tumor macrophages and abrogates glioma growth in mice. Glia 2009;57(13): 1458–67.
102. Gabrilovich DI, Chen HL, Girgis KR, et al. Production of vascular endothelial growth factor by human tumors inhibits the functional maturation of dendritic cells. Nat Med 1996;2(10):1096–103.
103. Kortylewski M, Kujawski M, Wang T, et al. Inhibiting Stat3 signaling in the hematopoietic system elicits multicomponent antitumor immunity. Nat Med 2005;11(12):1314–21.
104. Darnell JE Jr. STATs and gene regulation. Science 1997;277(5332):1630–5.
105. Raizer JJ. HER1/EGFR tyrosine kinase inhibitors for the treatment of glioblastoma multiforme. J Neurooncol 2005;74(1):77–86.
106. Salomon DS, Brandt R, Ciardiello F, et al. Epidermal growth factor-related peptides and their receptors in human malignancies. Crit Rev Oncol Hematol 1995;19(3):183–232.
107. Tang P, Steck PA, Yung WK. The autocrine loop of TGF-alpha/EGFR and brain tumors. J Neurooncol 1997;35(3):303–14.
108. Schaefer LK, Ren Z, Fuller GN, et al. Constitutive activation of Stat3alpha in brain tumors: localization to tumor endothelial cells and activation by the endothelial tyrosine kinase receptor (VEGFR-2). Oncogene 2002;21(13):2058–65.
109. Yu H, Jove R. The STATs of cancer–new molecular targets come of age. Nat Rev Cancer 2004;4(2): 97–105.
110. Carpentier AF, Xie J, Mokhtari K, et al. Successful treatment of intracranial gliomas in rat by oligodeoxynucleotides containing CpG motifs. Clin Cancer Res 2000;6(6):2469–73.
111. Carpentier AF, Auf G, Delattre JY. CpG-oligonucleotides for cancer immunotherapy: review of the literature and potential applications in malignant glioma. Front Biosci 2003;8:e115–27.
112. Auf G, Carpentier AF, Chen L, et al. Implication of macrophages in tumor rejection induced by CpG-oligodeoxynucleotides without antigen. Clin Cancer Res 2001;7(11):3540–3.