Review

Regulating Factors for Microglial Activation

Yoichi NAKAMURA

Laboratory of Integrative Physiology in Veterinary Sciences, Osaka Prefecture University; 1–1 Gakuen-cho, Sakai, Osaka 599–8531, Japan. Received January 10, 2002

Microglia, residential macrophages in the central nervous system, can release a variety of factors including cytokines, chemokines, *etc*. to regulate the communication among neuronal and other types of glial cells. Microglia play immunological roles in mechanisms underlying the phagocytosis of invading microorganisms and removal of dead or damaged cells. When microglia are hyperactivated due to a certain pathological imbalance, they may cause neuronal degeneration. Pathological activation of microglia has been reported in a wide range of conditions such as cerebral ischemia, Alzheimer's disease, prion diseases, multiple sclerosis, AIDS dementia, and others. Nearly 5000 papers on microglia can be retrieved on the Web site PubMed at present (November 2001) and half of them were published within the past 5 years. Although it is not possible to read each paper in detail, as many factors as possible affecting microglial functions in *in vitro* culture systems are presented in this review. The factors are separated into "activators" and "inhibitors," although it is difficult to classify many of them. An overview on these factors may help in the development of a new strategy for the treatment of various neurodegenerative diseases.

Key words microglia; pathological activation; neurodegenerative disease

1. INTRODUCTION

In the central nervous system (CNS), there are three kinds of glial cell other than neurons: astrocytes, oligodendrocytes, and microglia. These cells are surrounded by capillary endothelial cells and ependimal cells. The endothelial cells form a blood–brain barrier (BBB) that isolates central cells from peripheral tissues by maintaining highly stable conditions within the CNS. The immunological mechanism in the CNS was believed to be different from the peripheral tissues (so-called immunological privilege) due to the impermeability of the BBB, which prevents the entrance of immunoglobulins and the invasion of leukocytes from blood. However, many immunocompetent molecules have recently been identified within the CNS, including various cytokines and chemokines and their receptors. These immunological molecules are likely involved in CNS pathogenesis.

In the process of neuronal degeneration and thereafter, various phenomena occur. In the past, it was a general understanding that microglia (brain macrophages) are consequently activated after neuronal damage to phagocytose the damaged neurons, after which the postneuronal space is occupied by the proliferation of reactive astrocytes (gliosis or astrocyte scarring). However, Morioka *et al.*[1] observed that the microglial activation began prior to the neuronal degeneration in an animal model of transient brain ischemia. Since then, the possibility has been examined in various experimental systems that "microglial activation" is a cause of neuronal degeneration rather than a consequence of it (Fig. 1).

Under physiological conditions, residential microglia are quiescent and scattered throughout the CNS. Occasionally microglia are moderately activated to play the classic role as "scavengers" for the maintenance and restoration of the CNS. They begin to proliferate, changing their morphology into an amoeboid shape and phagocytose cells that are pathologically damaged or developmentally unnecessary. These functions of microglia are controlled by communication with cytokines, chemokines, trophic factors, and other neuromodulating molecules among neurons, astrocytes, and microglia. The true initial trigger(s) has not been established for microglial activation; however, some cytokines promote further microglial activation and others inhibit it. Such well-balanced microglial activation should be reversible and does not cause secondary neuronal degeneration. However, imbalanced microglial activation or hyperactivation of microglia may cause neurodegeneration. In the CNS where the milieu is well maintained due to the BBB, such an imbalance results from impairment of the BBB.

2. MICROGLIAL ACTIVATION IN VARIOUS NEUROLOGICAL DISEASES

It is well known that microglial cells play a key role in mediating inflammatory processes in the CNS, which are associated with various neurodegenerative diseases. Many epidemiologic studies have indicated that the use of antiinflammatory drugs reduces the incidence and slows the progress of Alzheimer's disease.[2] It is possible that the target of such antiinflammatory drugs is microglia, the major immunocompetent cells in the CNS. Growing evidence indicates that amyloid deposition and phagocyte activation participate in inflammatory reactions in the Alzheimer's brain. In AIDS dementia patients, progressive neurodegeneration is a consequence of the activation of microglia that are infected with HIV. In Huntington's disease, microglial overproduction of complements may cause neurodegeneration by their own activation.[3] Nitric oxide (NO) produced by microglia plays an important role in the death of dopaminergic neurons in MPTP model of Parkinson's disease.[4] In multiple sclerosis, the rapid destruction of oligodendrocytes by necrosis is followed by delayed destruction by apoptosis with an activated microglia/macrophage invasion.[5] Microglia also play important roles in the pathogenesis of spongiform encephalopathies (prion diseases).[6]

In addition to the above progressive neurodegeneration in the CNS, microglial activation is proposed to be involved in the secondary damage following the primary damage after traumatic brain injury.[7] Microglial activation is also believed

e-mail: yoichi@vet.osakafu-u.ac.jp

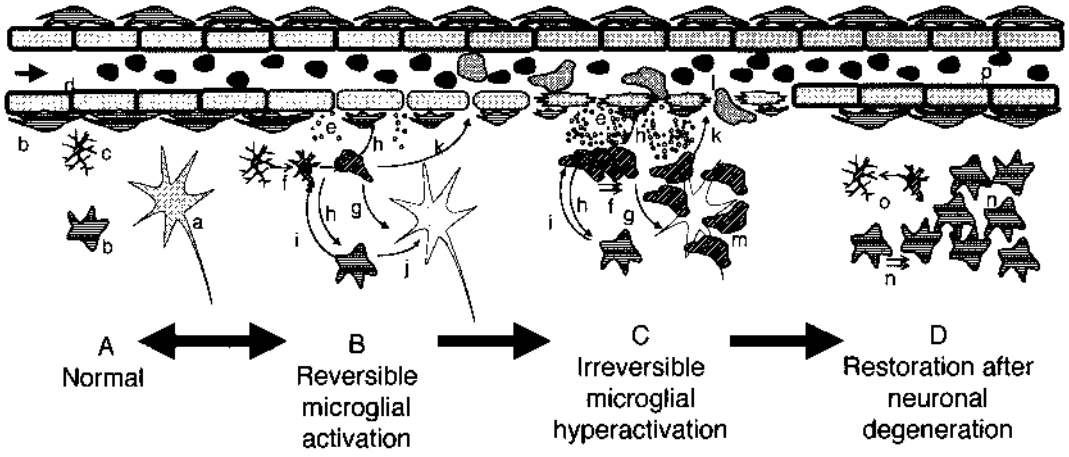

Fig. 1. Mechanism for Neuronal Degeneration by Microglial Activation

Stage A—Normal—a: Neurons. b: Astrocytes play roles in the maintenance of BBB impermeability and in the functions of neurotransmitter transport and metabolism around synaptic sites. c: Microglia (so-called ramified shape) possess many fine multiple-branched processes in the normal state *in situ.* d: Endothelial cells construct the BBB forming tight junctions with each other.

Stage B—Reversible microglial activation—e: Leaking of serum factors promotes microglial activation. f: Microglia start to proliferate, changing morphology into an amoeboid shape. g: Activated microglia produce substances causing neuronal injury, such as TNFα, NO, and O_2^-. h: Activated microglia also produce various cytokines, such as IL-1β and IL-6, affecting astrocyte functions. i: Astrocytes produce various factors, such as MIP1α. j: Astrocytes produce neuroprotective factors such as NGF. k: A microglial cytokine, such as TNF-α, affects endothelial cells, causing impairment of the BBB.

Stage C—Irreversible microglial hyperactivation—Responses e and k in stage B become significant in a synergistic manner; consequently responses f, g, and h are no longer controlled by physiological regulation. l: Invasion of blood cells occurs through breakdown of the BBB. m: Activated microglia and invading monocytes (macrophages) injure neurons.

Stage D—Restoration after neuronal degeneration—n: Astrocytes proliferate to occupy the postneuronal space (gliosis or glial scarring). o: Microglial activation is terminated (through apoptosis?) and the morphology returns to the ramified type. p: Capillary endothelium and surrounding astrocytes are repaired and BBB functions are restored.

to play an important role in pathological pain mechanisms such as nociceptive abnormality and hyperalgesia.[8] Even in psychiatric illnesses, it is reported that mononuclear phagocytes accumulate in the cerebrospinal fluid of schizophrenic patients during acute psychotic episodes,[9] and microglial activation is often observed in the postmortem pathohistologic examination of patients with schizophrenia.[10] In addition to the CNS, potential participation of microglial activation is also reported in glaucomatous optic nerve degeneration[11] and diabetic retinopathy.[12]

3. MICROGLIAL ACTIVATORS IN CULTURE

As mentioned above, numerous papers have been published on microglia. Table 1 is a list of molecules from such publications that simulate or enhance microglial activation mainly in *in vitro* cell culture experiments, and also a summary of the following text.

3.1. Conventional or Orthodox Activators

3.1.1. Lipopolysaccharide For the elucidation of the mechanism of microglial activation, many attempts have been reported using cell culture systems to specify a variety of factors in the rather complicated mechanism. Lipopolysaccharide (LPS) can trigger series of inflammatory reactions in phagocytes such as macrophages. LPS is a glycolipid derived from the membrane surface of gramnegative bacteria (endotoxin). In *in vitro* experiments using cultured microglia, LPS has generally been used for cell activation. With LPS stimulation microglia are activated to change their cellular functions drastically, producing various types of inflammatory cytokine, chemokine, and prostaglandin. In addition, LPS stimulates inducible nitric oxide synthase (iNOS) and NO production. In some pathologic conditions when the brain tissue is infected (meningitis) and also when the BBB is disrupted, microglia can be activated by encountering the bacterial cell wall; although the opportunities for such encounters are not frequent. LPS is often used to stimulate microglia for full activation in *in vitro* experiments.

The time course of LPS-induced activation of microglia is of interest. We demonstrated that the release of cytokines and NO showed different time courses after stimulation by LPS. First, tumor necrosis factor (TNF) α was released with 1-h lag time, second interleukin (IL)-1β within 3 h and, third IL-6. Finally, NO is released with about a 6-h lag time, and linear NO production continues for more than 48 h.[13] A marked morphologic change is also observed: upon the addition of LPS, a change from an amoeboid to a bipolar rod shape occurs for 3 to 6 h and then returns gradually to a small round shape.[13]

Before the release of cytokines, various cellular changes occur upon LPS addition. The mRNA level of myristoylated alanine-rich C kinase substrate (MARCKS) and MARCKS-related protein (MRP) increase with the increase in protein kinase C (PKC).[14] Prior to iNOS induction, activation of nuclear factor κB (NFκB) is necessary. It is reported that LPS induces the release of a large amount of pro-IL-1β other than mature IL-1β, although the role of pro-IL-1β is unknown.[15] Many other substances also have been reported to be released, including the cytokine IL-12p40,[16] various chemokines including MIP-1α and RANTES (for a review see ref. 17), histamine,[18] *etc.*

Changes in cellular enzymatic level have also been reported: iNOS induction with LPS stimulation is accompanied by the induction of argininosuccinate synthase and cationic amino acid transporter-2.[19] In addition, LPS stimulation results in the induction of cyclooxygenase 2 (COX2) and the production of prostaglandins, as discussed below.

3.1.2. Interferon γ (IFNγ) IFNγ has long been used as

Table 1. Stimulators and Enhancers of Microglial Activation

Activators	Production or Responses: enhancing (↑) or inhibiting (↓) (Ref.)
——Conventional stimulators——	
Lipopolysaccharide (LPS)	TNFα, IL-1β, IL-6, iNOS/NO, chemokines (MIP-1α) (13 *etc.*) BDNF, NGF (136)
Interferon γ (IFNγ)	↑CD40 expression (35) ↑LPS-induced NO, TNFα, ↑PMA-induced O_2^- ∗Fas/FasL-mediated microglial apoptosis (123)
LPS/INFγ	∗Microglialapoptosis (125)
Phorbol ester (PMA)	O_2^- ↓PGE_2-induced cAMP accumulation (90) ↑LPS-induced BDNF (136)
PMA (at low conc.)	↑Proliferation
——Senileplaquerelated molecules——	
β-Amyloid peptide (Aβ)	O_2^- (28) Outward rectifying K channel (29) Glutamate release (31) Ca^{2+} elevation (30) CD40-CD40L ligation (35) ↑TNFα-dependent iNOS (137) ↑LPS-induced IL-1β (27) ↓LPS-induced PGE, COX2 (138)
Aβ/IFNγ	IL-1β, MIP-1, NO (26 *etc.*)
Soluble APP	Glutamate release (32)
Amylin, amyloidotic peptide	↑LPS-induced IL-1β (47)
Chromogranin A	iNOS/NO (43 *etc.*), Cathepsin B release (46) ∗Microglial apoptosis (145)
Complement 1q	↑Phagocytosis (41) or ↓Phagocytosis (42)
——Prion related molecules——	
Prion protein fragment (PrP106—126)	IL-1β, IL-6, TNFα, iNOS/NO (49, 139) Ca^{2+} elevation (30)
——HIV-related molecules——	
Tat (HIV nuclear protein)	iNOS/NO, cytokines (50), chemokine (51)
Tat	Ca^{2+} elevation (52)
gp41 (HIV coat protein)	Cytokines, chemokines (51)
——Cytokines——	
CD40 ligand (CD154)	NO, TNF-α (38, 39)
IL-12 p40	iNOS/NO (53)
IL1β	Ca^{2+} elevation (55)
IL6	↑Proliferation (54)
——Other endogenous molecules——	
ATP	iNOS/NO, TNFα, IL-1β, IL-6 (57, 58, 59, 60 *etc.*)
Ganglioside	NO, TNFα, COX2. (73)
Thrombin	NO, TNFα, IL-6, IL-12, Ca^{2+} mobilization, proliferation (64, 65)
α-Crystallin (small heat shock protein)	iNOS/NO, TNFα (74)
Albumin	↑PMA-induced O_2^- (66, 67)
Mn^{2+}	↑LPS-induced iNOS/NO (68)
High K+	↑LPS-induced NO, TNFα (69)
LBP (LPS binding protein)	↑LPS-induced NO, TNF-α (66)
S100B (astrocyte derived protein)	↑Lipid A-induced NO (75)
GM-CSF	↑Proliferation (140)
Lysophosphatidic acid (LPA)	Ca^{2+} elevation (76)
Endotheline	Ca^{2+} elevation (77)
Platelet-activating factor (PAF)	Ca^{2+} elevation (78)

∗ Not simple activation (see text).

a co-stimulator with LPS by some researchers. In the presence of IFNγ most LPS responses are augmented. However, in our experience, IFNγ-augmentation is not always observed consistently, suggesting that the serum used in the culture medium often contains IFNγ-like factors.

3.1.3. Phorbol 12-Myristate 13-Acetate (PMA) PMA is another important activating factor used in experiments and is commonly used for the activation of PKC in various experimental cell systems. At very low concentrations (<1 nM), PMA promotes cell proliferation. It is known that macrophages collected from peritoneal fluid have differentiated and their proliferation is rarely detected, although cultured microglia collected from the neonatal rodent brain are in a proliferative stage under the usual culture conditions when media contain fetal bovine serum. The proliferation rate decreases with the removal of serum, and the cell proliferation is inhibited by PKC inhibitors, suggesting that the serum may contain a factor promoting microglial proliferation like granulocyte-macrophage colony stimulating factor (GM-CSF).

At higher concentrations of around 100 nM, PMA induces the production of superoxide (O_2^-). The most potent O_2^--generating cells are neutrophils, in which the enzyme(s) responsible for O_2^- generation have been identified as NADPH oxidase, a complex of gp91-phox and p22-phox with several regulatory members such as Rac, p67-phox, p47-phox, and

P40-phox. Neutrophils start O_2^- generation, the so-called respiratory burst, upon stimulation with opsonized zymosan, formyl-methionyl-leucyl-phenylalanine (fMLP), or PMA. In cultured microglia, only PMA has stimulatory activity, and the O_2^- production rate is lower than that of neutrophils (less than 1/10 on a cell number basis). gp91-phox is immunohistochemically detected in microglia in the brain[20] and p47-phox is detected cultured microglia.[21] Microglia contain a similar NADPH oxidase system, which includes a trigger with PKC activation, but it may not be identical to the system in neutrophils.

Expression of various isoforms of PKC have been demonstrated by Western blot analysis in cultured microglia: PKC βII, δ, θ, η, ζ, and ι are abundant and βI is less, but α, γ, and ε are absent. This expression pattern is different from that of astrocytes and peritoneal macrophages.[22] One of these isoforms plays a key role in triggering O_2^- generation. The other PKC isoforms play roles in the regulation of other cell functions. Different PKC inhibitors have different effects on microglial activation. For example, Gö6976 inhibits NO production but staurosporine enhances it.[23,24] Various protein kinases affect each other, that is, "cross talk" of intracellular signaling occurs.

3.2. Senile Plaque-Related Molecules

3.2.1. β-Amyloid Protein (Aβ) A major component of senile plaques in the Alzheimer's brain is Aβ. Important roles of microglia in the pathogenesis of Alzheimer's disease have been recognized since the direct effect of the Aβ peptide on microglia was demonstrated. Meda *et al.*[25] reported that the fragment Aβ [25—35] triggers the release of NO and TNFα from cultured microglia. Aβ-stimulated microglia also release IL-1β.[26,27] Aβ peptides stimulate the production of O_2^- and the production is inhibited by inhibitors of tyrosine kinases or phosphatidylinositol 3-kinase and by dibutyryl cAMP (dbcAMP), and is potentiated by IFNγ or TNFα.[28] Aβ peptides have other activity in addition to microglial activation, such as inducing the outward rectifying K channel[29] and elevating intracellular Ca^{2+} level.[30] Furthermore, Aβ peptide[31] and soluble amyloid precursor protein (sAPP)[32] enhance glutamate release by cystine exchange as a consequence of autoprotective antioxidant glutathione production within the microglia, ultimately causing synaptic degeneration and neuronal death. The intracellular signaling pathway of Aβ stimulation is likely to be similar to that of PKC activation, involving MARCKS.[33]

Ligation between CD40 and its ligand (CD40L) plays a critical role in the mechanism of Aβ-stimulation.[34] CD40 expression on microglia is greatly enhanced by IFNγ[35,36] and is inhibited by IL-4.[37] The ligation of microglial CD40 with CD40L triggers significant production of TNFα[35] through the p44/42 MAPK pathway,[38] an expression of iNOS through NFκB activation.[39]

3.2.2. Complement Components Within the senile plaques in Alzheimer's disease, the complement component C1q is co-localized with fibrillar Aβ.[40] C1q modulates the phagocytotic activity of microglia. Two controversial roles of C1q have been reported: C1q enhances phagocytosis of Aβ[41] and inhibits it.[42]

3.2.3. Chromogranin A Chromogranin A, a multifunctional protein, is localized in dystrophic neurites and in senile plaques. The direct effect of chromogranin A was demonstrated in cultured microglia where it induced a morphologic change to a flattened amoeboid shape, rapidly elevated intracellular Ca elevation, and induced NO generation comparable to that induced by LPS[43] with subsequent neuronal injury.[44] Chromogranin A-activated microglia can trigger neuronal apoptosis mediated by the secretion of several death-signaling pathways[45] including cathepsin B.[46]

3.2.4. Amylin Amylin, another amyloidotic peptide, is also reported to enhance microglial activation. In microglial culture, LPS-induced IL-1β production was enhanced by amylin.[47] Microglia are able to response to various fibrous proteins, as described above. Microglia may have the ability to detect a fibrous configuration by an unknown mechanism, rather than a specific structure formed by the respective peptide sequences.

3.3. Prion Protein Prion, the transmissible proteinous agent of bovine spongiform encephalopathy and human Creutzfeldt-Jacob disease, should play a key role in the pathogenesis of such progressive neurodegenerative diseases. Amyloidotic deposits are also seen pathohistologically in addition to numerous vacuoles in the neurodegenerative area. The deposits are composed of abnormal prion protein (PrPSc). The pathogenic mechanism has not been fully elucidated, although PrPSc is believed to be a key molecule. It has been demonstrated that prion protein fragment (PrP106—126) is neurotoxic[48] and induces microglial activation of iNOS expression and TNFα production.[49] Activation of microglial cells by PrP106—126 results in elevated intracellular Ca^{2+} levels through L-type voltage-sensitive Ca channels, which subsequently produces neurodegeneration.[30]

3.4. HIV-Related Molecules In AIDS dementia patients, the progressive neurodegeneration is the consequence of microglial activation that is infected with HIV-1. Tat (an HIV nuclear protein) and gp41 (an HIV coat protein) both stimulate iNOS expression and NO production in microglial cultures[50] and the release of cytokines and chemokines.[51] Tat triggers intracellular Ca^{2+} elevation[51] and TNFα production.[52]

3.5. Cytokines LPS-induced microglia produce various kinds of proinflammatory cytokines, as mentioned above. Those cytokines themselves affect microglia. IL-12p40 triggers microglial iNOS expression and NO release.[53] Exposure to IL-6 stimulates microglial proliferation.[54] IL-1β induces the elevation of intracellular Ca^{2+} levels *via* the dual pathways of Ca^{2+} entry and Ca^{2+} mobilization.[55]

3.6. ATP Recently, there have been various reports on the purinergic mechanism in the regulation of cellular function in the CNS. Microglia express several types of purinergic receptors including the metabotropic (P2Y) and ionotropic (P2X) types.[56] ATP has various direct effects on cultured microglia. Even in the absence of LPS, ATP acts on P2 receptors and induces TNFα release,[57] IL-1β release,[58] iNOS expression with NO production,[59] and IL6 release.[60] In addition, ATP acting on PTX-insensitive P2Y receptors activates p38 and Ca^{2+}-dependent PKC, thereby resulting in the mRNA expression and release of IL-6 in the microglial cell line MG-5.[60] These responses resemble those when the cells are stimulated by LPS.

ATP also elevates intracellular Ca^{2+} levels. The percentages of cells responding to ATP differs between those with (50%) and without (85%) treatment with LPS.[61] Receptor

expression may change with LPS treatment. Cultured rat retinal microglia expresses metabotropic P2U (P2Y2, P2Y4) and ionotropic P2Z (P2X7) equally, whereas in LPS-stimulated microglia, P2Z dominates.[56] Continuous activation of P2Z/P2X7 purinoreceptor induces microglial cell death by apoptosis.[62] Purinergic stimulation results in membrane ruffling and chemotaxis of microglia, which are mediated by G(i/o)-coupled P2Y receptors.[63]

3.7. Serum Factors It is interesting that the serum protein thrombin has the ability to stimulate microglia. Thrombin can induce the production of TNFα, IL-6, IL-12,[64] and NO.[65] In addition to such responses similar to that with LPS, Ca^{2+} mobilization and cell proliferation are also induced.[64]

LPS-induced production of TNFα and NO is augmented in the presence of serum.[66] The 60-kDa protein responsible for the augmentation is strongly suggested to be LPS binding protein (LBP). The most abundant protein in serum, albumin, also shows an effect: PMA-induced O_2^- production is potentiated by the presence of albumin at concentrations of less than 50 μg/ml (<1 μM).[66] Trypsin-treated albumin still maintains the potentiating activity, and the active subfragment of albumin has been purified. The minimum structure for such potentiating activity was the four amino acid peptide Leu-His-Thr-Leu.[67] The BBB impermeability is not always well maintained. The BBB is often broken down under certain pathological conditions. Various serum factors leaking into the central parenchyma should affect microglial cell functions and consequently aggravate neuronal damage.

3.8. Other Endogenous Molecules The effects of metals or salts are also interesting. Manganese increases LPS-induced NO production along with the iNOS mRNA level. The effect is not mimicked by the other transition metals iron, cobalt, nickel, copper, and zinc.[68]

In the presence of high concentrations (20—40 mM) of KCl, LPS-induced reactions of microglia are enhanced. NO and TNFα production[69] and iNOS expression increase, and K^+-channel blockers inhibit (our unpublished results). These fairly long-term effects (more than 1 d) may be due to changes in intracellular K^+ and/or Cl^- concentrations. Several types of K^+ channel have been identified on the microglial cell membrane. A switch in their expression causes a change in the cell membrane potential.[70] Their differential expression is responsible for cell functions including cell proliferation.[71] Treatment with LPS also affects K^+ channel expression, and the expression can be modified by varying the cAMP level.[72] Some other endogenous factors have also been reported to trigger microglial activation.

Ganglioside can induce TNFα and COX2 expression and NO production.[73] α-Crystalline, a small heat-shock protein, stimulates microglia to produce TNFα and NO.[74] S100B, an astrocyte-derived protein, also augments NO production from lipid A-stimulated microglia.[75] The intracellular Ca^{2+} concentration is a ubiquitous regulatory signal used for cell activation in almost all animal cells. Several endogenous molecules, lysophosphatidic acid (LPA),[76] endothelin,[77] platelet-activating factor (PAF),[78] *etc.*, have been shown to elevate intracellular Ca^{2+} levels in cultured microglia.

4. INHIBITORS OF MICROGLIAL ACTIVATION

Table 2 is a list of molecules that inhibit microglial activation mainly in *in vitro* cell culture experiment, and also a summary of the following text.

4.1. cAMP-Related Molecules Intracellular cAMP level is a key factor in a variety of cell functions. The direct effect of cAMP can be detected by the addition of cell membrane-permeable cAMP analogs, such as dbcAMP and 8Br-cAMP. In the case of cultured microglia, cAMP reduces cellular functions in general. dbcAMP inhibits cell proliferation and PMA-induced O_2^- production. In addition, LPS-stimulated production of TNFα and IL-1β is also inhibited. However, it should be noted that dbcAMP does not affect the LPS-induced production of IL-6 and NO.[79] On the contrary, dbcAMP is reported to enhance Aβ-stimulated NO production.[80]

Some other reagents that increase cAMP levels also have inhibitory effects. Phosphodiesterase inhibitors, such as propentofylline[79] and ibudilast,[81] inhibit LPS-induced TNFα production. Vasoactive intestinal peptide (VIP) and its related peptide, pituitary adenylyl cyclase-activating polypeptide (PACAP) show inhibitory effects on LPS-induced TNFα expression.[82]

4.2. Prostaglandins In a series of responses after LPS stimulation, COX2 expression[83] and consequent production of prostaglandin E_2 (PGE_2)[84] are important for autoregulation of microglia, that is, deactivation. Activation of microglia leads to the production of prostaglandins, similar to the general proinflammatory response, although the addition of exogenous PGE_2 inhibits the LPS-induced production of NO and cytokines. The signaling mechanism of this effect is likely *via* cAMP.[85] A potent antiinflammatory action of 15-deoxy-Δ(12,14)-prostaglandin J(2) on microglia has been reported. It inhibits LPS-induced iNOS induction[86] and LPS-stimulated production of TNFα, IL-1β, COX2,[87] and IL-12.[88] Cross-regulatory effects of IFNγ and PGE_2 have been demonstrated in the LPS-induced production of the pro- and antiinflammatory cytokines TNFα and IL-10, respectively. INFγ enhances TNFα but inhibits IL-10 production and PGE_2 has the opposite effects.[89]

The receptor responsible for these prostaglandin effects is likely to be EP_2, which is coupled to Gs protein leading to cAMP accumulation. PMA reduces the PGE_2-induced cAMP accumulation, provably *via* EP_2 phosphorylation by a PKC, and prevents the cAMP-mediated deactivation of microglia.[90]

4.3. Steroids The estrogen 17β-estradiol inhibits the LPS-induced production of the inflammatory mediators iNOS, PGE_2, and metalloproteinase-9 (MMP-9) in primary microglia,[91] superoxide release and phagocytic activity in the microglial cell line N9,[92] and Tat-mediated microglial activation in N9.[93] Hydrocortisone and dexamethasone also inhibit LPS-induced microglial NO production in N9.[94] Lipocortin 1, a dexamethasone-induced protein, also decreases PGE_2 and NO productions.[95] All these effects are inhibitory against microglial activation, although two reports have shown apparent enhancing effects.[96,97] Aβ uptake in cultured microglia was enhanced by pretreatment with estrogen.[96] Dehydroepiandrosterone inhibits cell growth and induces apoptosis in BV-2 cells.[97]

4.4. Opioids and Their Antagonists Microglial responses to several opioids are also interesting. Endomorphins (μ-opioids) block phagocytosis and chemotaxis.[98] However,

Table 2. Inhibitors of Microglial Activation

Inhibitors	Production or responses: enhancing (↑) or inhibiting (↓) (Ref.)
——cAMP related molecules——	
cAMP (cell permeable)	↓LPS-induced TNFα, IL-1β, PMA-induced O_2^-, proliferation (13)
	↓LPS-induced IL-12p40 (16)
	∗↑Aβ-induced NO (80)
PDE inhibitors	↓LPS-induced TNFα (141)
Propentofylline (PDE inhibitor)	↓LPS-induced TNFα, IL-1β, PMA-induced O_2^-, proliferation (13)
Vasoactive intestinal peptide (VIP)	↓LPS-induced TNFα mRNA (82)
Pituitary adenylyl cyclase-activating polypeptide (PACAP)	↓LPS-induced TNFα mRNA (82)
Prostaglandine E_2 (PGE_2)	↓LPS-induced NO, TNFα, IL-1β (85)
	cAMP accumulation (90)
15-Deoxy-Δ (12,14)-PGJ_2	↓LPS-induced NO, TNFα, IL-1β (87)
——Steroids——	
Hydrocortisone	↓LPS-induced iNOS (94)
Dexamethasone (Lipocortin-1)	↓LPS-induced NO, PGE_2 (95)
Dehydroepiandrosterone (DHEA)	↓Microglial apoptosis (97)
17β-Estradiol	↓LPS-induced iNOS, PGE_2, MMP-9 (91, 92)
	↑Aβ uptake (96)
——Opioids——	
Endomorphines (μ-opioids)	↓Phagocytosis, chemotaxis (98, 99)
	∗↑PMA-induced O_2^- (99)
Naloxone (μ-antagonist)	↓PMA-induced O_2^- (100)
Naloxone (μ-antagonist) in mixed culture	↓LPS-induced NO, TNFα (102)
Dynorphin (κ-opioids) in mixed culture	↓LPS-induced neurotoxicity (103)
——Other endogenous molecules——	
Adenosine (2Cl-adenosine)	Microglial apoptosis (122)
Melatonine	↓Aβ-induced IL-1β, IL-6 (in brain slice) (111)
α-Melanocyte stimulating hormone (MSH)	↓Aβ/INFγ-induced NO/TNFα (110)
Apolipoprotein E	↓LPS-induced TNFα and NO (109)
IL10	↓LPS-induced IL-1β, TNFα, IL-2R, IL-6R (107)
Neurotrophins (NGF, BDNF, NT-3, NT-4)	↓LPS-induced NO (133)
	↓Urokinase type-plasminogen activator (uPA) (133)
Ceramide	↓Urokinase-type plasminogen activator (uPA) (142)
——Other exogenous molecules——	
Cannabinoids	↓LPS-induced mRNAs for IL-1α, IL-1β, IL-6, TNFα (112, 113)
N-Acetyl-*O*-methyldopamine (NAMDA)	↓LPS-induced mRNAs for IL-1β, TNFα, iNOS (115)
K252a (pyridazine-based CaMK inhibitor)	↓LPS-induced NO (118)
Atratoglaucosides	↓LPS-induced TNFα (116)
Thalidomide	↓LPS-induced chemokine (IL8) (117)
Minocycline (Tetracycline derivative)	↓NMDA-induced proliferation, NO, IL-1b (119)
Nicergoline	↓PMA or zymosan-induced O_2^- (120)
Diazepam (benzodiazepine)	↓Tat-induced Ca^{2+} elevation (106)
Thapsigargin	↓Transformation (keeping ramified shape) (143)
Agmatine (endogenous amine)	↓NOS activity (144)

∗ Not simple inhibition (see text).

endomorphins potentiate PMA-induced O_2^- production under certain conditions when microglia are briefly activated by LPS.[99] Naloxone (a favored μ-opioid antagonist) can reverse the effect of endomorphins[99] and PMA-induced O_2^- production.[100] Naloxone protects rat dopaminergic neurons against inflammatory injury through inhibition of microglial activation and superoxide generation.[101] Naloxone is reported to inhibit LPS-induced NO production and TNFα release in mixed culture, *via* nonclassic opioid receptors, because the usually ineffective enantiomer (+)-naloxone was equally effective.[102]

Other opioids also show some effects. Dynorphin (κ-opioid) reduces LPS-induced neurotoxicity in culture,[103] δ-like opioid peptide is released from embryonic mixed brain cells, promoting macrophage migration.[104]

4.5. Peripheral Benzodiazepine Receptor Benzodiazepines readily penetrate the BBB and are known to have antiinflammatory properties. The peripheral type of benzodiazepine receptor is used as a cell surface marker. *In vivo* detection of increased [^{11}C](R)-PK11195 binding occurs in the entorhinal, temporoparietal, and cingulate cortex in Alzheimer-type dementia, suggesting that microglial activation is an early event in the pathogenesis of the disease.[105]

Pretreatment of microglial cells with peripheral (Ro5-4864) and mixed (diazepam), but not central benzodiazepine receptor ligands was found to suppress in a potent manner Tat-induced chemotaxis due to blocking of Tat-induced Ca^{2+} mobilization.[106]

4.6. Other Endogenous Molecules Many other endogenous molecules are reported to inhibit microglial activation. The antiinflammatory cytokine IL-10 inhibits LPS-induced IL-1β and TNFα production.[107] Treatment with vitamin E reduces LPS-induced NO, IL-1α, and TNFα production.[108] Apolipoprotein E and its mimetic peptides downregulate the LPS-induced production of TNFα and NO.[109] α-Melanocyte-stimulating hormone (MSH) reduces Aβ-in-

duced production of NO and TNFα,[110] and melatonin inhibits Aβ-induced production of IL-1β and IL-6 in brain slices.[111]

4.7. Other Exogenous Molecules LPS-induced microglial activation is inhibited many other reagents, including cannabinoids,[112,113] celastrol (plant-derived triterpene),[114] *N*-acetyl-*O*-methyldopamine,[115] atratoglaucosides,[116] thalidomide,[117] K252a (pyridazine-based CaMK inhibitor),[118] minocycline (tetracycline derivative).[119] Nicergoline, commonly used in the treatment of chronic cerebral infarction, inhibits PMA-induced O_2^- production.[120]

5. MODULATION OF MICROGLIAL ACTIVATION

In the above sections, various factors affecting microglial activation or inhibition are described as separately as possible, although it is difficult to specify their effects in some cases. However, other factors modulate microglial cell functions without simply activating or inhibiting them.

5.1. Microglial Apoptosis An important process in microglial activation is sedation of the microglia to terminate various inflammatory responses. In culture, serum deprivation induces apoptotic cell death with the expression of Bax protein.[121] The addition of 2Cl-adenosine (a stable adenosine analogue) induces remarkable microglial apoptosis even in the presence of serum.[122]

Microglial activation on a large scale in the presence of IFNγ induces apoptosis and augments the expression of Fas and Fas ligand (FasL).[123] Interferon regulatory factor-1 and caspase-11 are the essential molecules in activation-induced cell death of microglial cells.[124] Overactivation-induced apoptosis of microglia is a fundamental self-regulatory mechanism. NO may be the major autocrine mediator in this process.[125] The Fas-mediated apoptosis of microglia is inhibited by transforming growth factor (TGF) β[126] and a chemokine, fractalkine.[127]

5.2. Glutamate Receptors and Transporters Several receptors for glutamate are localized on microglial cell membrane. Several subtypes of ionotropic glutamate receptor, GluR2-5, 7 and KA1-2, have been identified in microglia by electrophysiology, immunohistochemistry, and reverse-transcription-PCR examination. The activation of these receptors significantly enhances the production of TNFα.[128] The expression of mGlu5a receptor mRNA and the occurrence of 1*S*,3*R*-ACPD-induced calcium signaling were found also in cultured microglia.[129] It is reported that MK801, a specific NMDA antagonist, shows cytotoxicity for cultured microglia and that the toxicity was reduced by glutamate and NMDA[130] suggesting the occurrence of NMDA receptor.

Microglia possess glutamate transporters. GLT1 (EAAT2) but not GLAST (EAAT1) has been identified in microglia by immunologic and pharmacologic evidences.[131] A neuroprotective role of microglia is suggested by the finding that GLT-1 is highly expressed in activated microglia following facial nerve axotomy.[132] After treatment with Aβ peptide [25—35], an increase in both reverse and forward glutamate transport is observed in cultured microglia using electrophysiologic techniques, suggesting a possible cause of neuronal damage.[31]

6. CONCLUSION

Under physiologic conditions, resident microglia are quiescent and scattered throughout the CNS. Occasionally microglia are moderately activated to play a classic role as "scavenger" for the maintenance and restoration of the CNS. They begin to proliferate, changing morphology into an amoeboid shape, and phagocytose the cells that are pathologically damaged or developmentally unnecessary. These functions of microglia should be controlled by communication among neurons, astrocytes, and microglia with cytokines, chemokines, trophic factors, and other neuromodulating molecules. Well-balanced microglial activation should be reversible and does not cause secondary neuronal degeneration. However, imbalanced microglial activation or hyperactivation of microglia may cause neurodegeneration. Many candidates for the trigger of the imbalance have been proposed, as outlined in this review. To develop new strategies for the treatment of neurodegenerative diseases, we may find rational targets from among these regulating factors.

This review describes the factors within the range of "microglial activation." Most of the activation leads to neurotoxic consequences although some neurotrophic effects of microglia have also been reported. Microglia can produce various neurotrophic factors, such as brain-derived neurotrophic factors (BDNF) and glial cell line-derived neurotrophic factor (GDNF), and they may play a role in neuroprotection.[133,134] Stimulation of microglia with LPS increases the secretions of BDNF, nerve growth factor (NGF), and neurotrophin-3 (NT-3).[133] The most advantageous strategy for therapy should utilize these neuroprotective effects of microglia in addition to the blocking their neurotoxic effects.

An interesting epidemiologic study was reported recently. A prospective population-based cohort study of about 7000 individuals revealed that the relative risk of Alzheimer's disease decreases to 0.2 with the use of nonsteroidal anti-inflammatory drugs (NSAIDs) for more than 2 years.[135] Not only clinical therapies for neurodegenerative diseases, but also the drugs for prevention of such diseases are urgently needed.

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