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Microglia, major player in the brain inflammation: their roles in the pathogenesis of Parkinson's disease

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Abbreviations: Aβ, amyloid beta; AD, Alzheimer's disease; α-MSH, alpha-melanocyte stimulating hormone; BBB, blood brain barrier; COX-2, cyclooxygenase-2; IL-1Ra, IL-1 receptor antagonist; iNOS, inducible nitric oxide synthase; LBP, LPS-binding protein; MMP-3, matrix metalloproteinase-3; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; MS, multiple sclerosis; NADPHO, NADPH oxidase; NFTs, neurofibrillary tangles; NF-κB, nuclear factor κ B; NO, nitric oxide; PD, Parkinson's disease; RAGE, receptors for advanced glycosylated end-products; SN, substantia nigra; Tlr4, toll-like receptor 4; tPA, tissue plasminogen activator; VIP, vasoactive intestinal peptide

Abstract

Inflammation, a self-defensive reaction against various pathogenic stimuli, may become harmful self-damaging process. Increasing evidence has linked chronic inflammation to a number of neurodegenerative disorders including Alzheimer's disease (AD), Parkinson's disease (PD), and multiple sclerosis. In the central nervous system, microglia, the resident innate immune cells play major role in the inflammatory process. Although they form the first line of defense for the neural parenchyma, uncontrolled activation of microglia may directly toxic to neurons by releasing various substances such as inflammatory cytokines (IL-1β, TNF-α, IL-6), NO, PGE_2, and superoxide. Moreover, our recent study demonstrated that activated microglia phagocytose not only damaged cell debris but also neighboring intact cells. It further supports their active participation in self-perpetuating neuronal damaging cycles. In the following review, we discuss microglial responses to damaging neurons, known activators released from injured neurons and how microglia cause neuronal degeneration. In the last part, microglial activation and their role in PD are discussed in depth.

Keywords: inflammation; microglia; neurodegenerative diseases; Parkinson's disease; phagocytosis; stromelysin 1; superoxides

Introduction

Inflammation is the first response of our body's immune system to pathogens or irritation. Inflammation is a two-edged sword. In acute conditions, it protects tissue against invading agents and promotes healing. On the other hands, when chronically sustained, it can cause serious damage to host's own tissue. While the CNS has been known as an immune privileged organ, increasing evidence demonstrate that inflammation is actively involved in pathogenesis of a number of neurodegenerative diseases including multiple sclerosis (MS), Alzheimer's disease (AD), Parkinson's disease (PD), and HIV-associated dementia (Raine, 1994; Banati *et al.*, 1998; McGeer *et al.*, 1988). Chronic inflammation-mediated tissue damage can be particularly harmful to the brain, since neurons are generally irreplaceable. Microglia, antigen presenting brain immune cells (or macrophages), are the innate immune components in the CNS parenchyma. Under normal condition, they may sentinel the CNS parenchymal integrity. Activated microglia at the site of inflammation change their morphology, express increased levels of MHC antigens and become phagocytic (Hayes *et al.*, 1987; 1988). They release inflammatory cytokines that amplify the inflammatory response by activating and recruiting other cells to the brain lesion. In addition, microglia can release potent neurotoxins, which may cause neuronal damage. Sustained overactivation of microglia has been observed in multiple neurodegenerative diseases. In the following review, we mainly discuss the microglial activation and its implication in the pathogenesis of PD.

Unique immune properties of the central nervous system

Antigen presentation is an important process in the immune system which links innate immunity to adaptive immunity. This process involves APCs capable of engulfing foreign pathogens, processing and presenting antigen in the MHC molecules on the cell surface. Antigens on the surface of APCs are recognized either by CD4 or by CD8 T cells and they become fully activated only when receiving additional co-stimulatory signals from APCs. While most peripheral tissues are supported by immune surveillance coordinated by T cells and APCs such as

macrophage and dendritic cells (professional APCs) or B cells and certain stromal cells (non-professional APCs) (Mellman and Steinman, 2001), such sentinels seem limited in the CNS. In fact, allografts were demonstrated to survive longer than those on other tissues (Barker and Billingham, 1977). The blood brain barrier (BBB) consisting of endothelial tight junction, parenchymal basement membrane and glial limitans of astrocytes, restricts the passage of plasma proteins and the immune components such as lymphocytes, antibodies and complements. Furthermore, the CNS was thought to lack of conventional lymphatic drainage. It was reported that a number of anti-inflammatory substances such as TGF-β, Fas ligand and ganglioside, which inactivate or kill immune cells (Becher *et al.*, 1998; Benveniste, 1998; Irani, 1998; Bechmann *et al.*, 1999; Gozes *et al.*, 1999; Pender and Rist, 2001; Vitkovic *et al.*, 2001). Increasing reports, however, demonstrate that some degree of immune surveillance of the CNS exists and is crucial to prevent disease development. For instance, some latent viruses such as JC virus or varicella zoster produce clinical syndromes mainly in immuno-compromised individuals. MHC and co-stimulatory molecules are strongly increased in CNS infection and a number of CNS pathologic conditions, including ischemia, neoplasm, traumatic nerve injury and neurodegenerative diseases such as AD, PD, MS, HIV-dementia and Creutzfeldt-Jacob disease (Maehlen *et al.*, 1989; McGeer *et al.*, 1993; An *et al.*, 1996; Graeber *et al.*, 1998; Perry, 1998; Dorries, 2001; Piehl and Lidman, 2001; O'Keefe *et al.*, 2002; Owens, 2002). Recent evidence has implied that the CNS equips itself with a unique defense system with its own molecular and cellular components despite sharing certain properties with the peripheral immune system.

Cellular components involved in the inflammatory response associated with neurodegeneration comprise mainly two parts according to their location: resident cell types in the parenchyma such as astroglia and microglia; CNS-associated cells, phagocytes characterized as macrophages and dendritic cells based on molecular and functional phenotype. They have been recently identified in compartments associated with the CNS, such as the Virchow-Robin (perivascular) space, the leptomeninges, and the choroids plexus (McMenamin, 1999; Serafini *et al.*, 2000; Fischer and Reichmann, 2001; Williams *et al.*, 2001). CNS-associated cells are continuously replenished by bone-marrow derived cells (Hickey *et al.*, 1992; Bechmann *et al.*, 2001). Under various pathologic conditions of the CNS, they are activated and increase numbers. They constitutively express high levels of MHC II, CD11b, co-stimulatory molecules and leukocyte common antigen CD45 ($CD45^{high}$). In contrast, the levels of CD45 of parenchymal microglia are relatively low ($CD45^{low}$). Isolated these $CD45^{high}$ CNS-associated cells showed to activate both naïve and primed CD4 T cells even more efficiently than $CD45^{low}$ microglia isolated from the same animals (Ford *et al.*, 1996; Carson *et al.*, 1998; 1999).

Microglia: the immune component in the CNS parenchyma

The initial definitive investigation of microglia was carried out by del Rio-Hortega early in the 20^{th} century. His intensive research described microglia as a distinct cell type in the central nervous system with extensively branched morphology and close contact with neurons and astrocytes. He also showed that the cells changed their morphology dramatically upon severe brain injuries such as stab wounds and that they migrated to the lesion site, proliferated and phagocytosed dying cells and other debris (del Rio-Hortega, 1932). Microglia comprise about 10% of the total glial population in the CNS parenchyma (Perry, 1998). Regarding the origin of microglia, the most widely accepted hypothesis is that they migrate from yolk sac to the CNS and differentiated into microglia (Alliot *et al.*, 1999). Microglia, however, are not readily differentiated from bone marrow-derived monocytes during adulthood, suggesting they derive from different monocytic leanage (Hickey *et al.*, 1992; Becher *et al.*, 2003). Microglia are important to the normal development of the embryo. Overproduced neurons destined to die by programmed cell death are thought to be removed by microglia that display an immature, nonramified morphology. After the completion of the CNS sculpting, microglia fully differentiate into a ramified resting state. The function of resting microglia has not been fully elucidated, but they may release low levels of growth factors that support the survival of neurons and glia. Recent *in vivo* imaging studies demonstrate that microglia actively monitor the CNS parenchymal environment by continual movement of their fine processes in the healthy brain (Nimmerjahn *et al.*, 2005). In the mouse brain cortex, microglial processes and protrusions directly contact to astrycytes, neuronal cell bodies and blood vessel, suggesting close communication each others (Nimmerjahn *et al.*, 2005). Upon focal stresses, microglial processes extend toward the injured sites and shield the injured area from the healthy tissue. It is also suggested that ATP released from the damaged neurons or astrocytes regulates microglial process dynamics and rapid response towards injury (Davalos *et al.*, 2005).

These results indicate that they form the first line of defense against brain damage and play a role in maintaining the microenvironmental homeostasis by removing dead cells and microbes and by supporting damaged neurons. However, by excessive releasing of substances which may be dangerous to cells, microglia might contribute to neurologic disorders (Streit and Kincaid-Colton, 1995). Overall the effect of microglia may depend on both pathologic conditions and severity of injuries.

Neuronal control of microglial quiescence under normal conditions

Recent studies have showed that neurons suppress microglial activation in coordinate with astrocytes under normal physiologic conditions of the CNS. A glycoprotein, CD200 expressed on the surface of neurons, keeps microglia quiescence by engagement with its receptor on microglia (Hoek *et al.*, 2000). In CD200 knock out mice, microglia were spontaneously activated as determined by increased CD11b and CD45 as well as by loss of their ramified morphology (Hoek *et al.*, 2000). Electrical activity and soluble factors released from intact neurons also maintain microglial quiescence. In neuron-glia co-culture, blockade of neuronal electrical activity by tetrodotoxin or glutamate receptor antagonist facilitated microglial activation induced by IFN-γ (Neumann, 2001). The soluble molecules from neurons including neurotransmitters and trophic factors also led to suppression of MHC II and co-stimulatory molecules for antigen presentation (Wei and Jonakait, 1999; Neumann, 2001). Interestingly, astrocytes are able to suppress microglial activation by releasing TGF-β or IL-10 (Vincent *et al.*, 1997; Aloisi, 2001) (Figure 1). Taken together, under normal physiologic conditions, microglia are maintained quiescence by coordinate action of neurons and astrocytes. When integrity of the CNS parenchyma is disrupted, microglia are rapidly activated, probably as results of both loss of inhibition by neurons or direct activation signal from neurons (Figure 2). The signals involved in microglial activation will be discussed in detail later.

Antigen presentation by Microglia

Microglia are the only resident cells in the CNS that have the capability to mediate immune responses. Their antigen presentation *in vivo* has been demonstrated both in the normal brain and inflammatory conditions caused by LPS injection, neuronal damage or MS lesions (Andersson *et al.*, 1992; Shrikant and Benveniste, 1996; Krogsgaard *et al.*, 2000). To fully activate T-cells, microglia are required to express HLA/MHC with members of the B7 and/or CD40 families, the co-stimulatory molecules. The expression of B7-1, B7-2, B7-3 and CD40 mRNA are shown in rat microglia, and are up-regulated by GM-CSF. Cytokines and growth factor-mediated regulation of these molecules have been demonstrated. A MHC class II molecule is important to antigen presentation. The expression of this molecule in microglia can be regulated by a variety of agents. IFN-γ is a potent stimulant of MHC class II antigens (Pazmany *et al.*, 2000). Upregualtion of MHC class II, CD40 and ICAM-1 by IFN-γ triggers T-cell proliferation and production of IL-2 and IFN-γ by Th1 and IL-4 by Th2 cells (Aloisi *et al.*, 1998). Several molecules including PGE2, neurotrophins, IL-10 and TGF-β1 have also been reported as suppressors of MHC class II antigens (Suzumura *et al.*, 1993; Levi *et al.*, 1998; Neumann *et al.*, 1998; Broderick *et al.*, 2000). This body of evidence delineates microglia as the specific cells which have the capacity for professional antigen presentation.

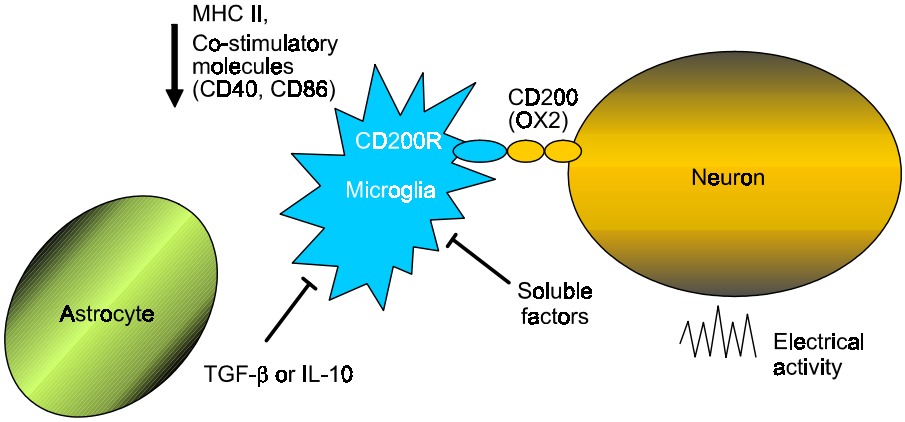

Figure 1. Maintenance of microglial quiescence under normal physiologic condition of the CNS. Microglia are suppressed by neurons or astrocytes under normal condition. Microglial activation is inhibited by the engagement of CD200, glycoprotein expressed on the surface of neurons and its receptor on microglia. Normal electric activity and soluble factors released from neurons (neurotransmitters and neurotrophic factors) also suppress microglial MHC II and co-stimulatory factors such as CD40 and CD86. TGF-β and IL-10 secreted from astrocytes also inhibit microglial activation.

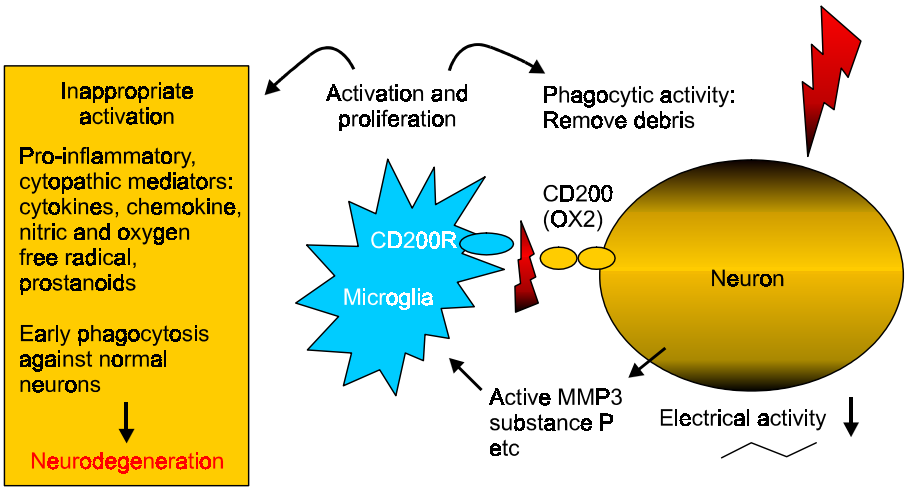

Figure 2. Microglial activation upon neuronal damage. When neurons are injured, microglia are rapidly activated as a result of both a weakening neuronal inhibitory signals and activating stimulatory signals. Loss of neuronal integrity results in breakdown of microglial inhibitory molecules such as CD200, soluble inhibitors. Instead, microglial stimulatory signals including MMP-3 and substance P are rapidly turned on and activate microglia. Activated microglia phagocytose at sites of neuronal damage, and increase in numbers, either from proliferation or recruitment, in order to remove damaged tissue and promote repair. However, their inappropriate activation as in neurodegenerative disorders causes direct damage to the CNS either by releasing potentially toxic substances such as proinflammatory cytokines, chemokines, NO, superoxide and prostanoids or by early phagocytosis against normal neurons.

Antiinflammatory and proinflammatory cytokine release by Microglia

In the normal CNS, brain tissue provides an immunosuppressive environment, which seems to be important for proper function of the CNS. This immunosuppressive environment is supported by several molecules such as TGF-β, α-MSH, VIP and FasL, and also by the BBB (Cserr and Knopf, 1992; Perry, 1998; Bechmann *et al.*, 1999; Flugel *et al.*, 2000). Under circumstances that cause the disruption of this environment, such as infectious diseases, autoimmune diseases or chronic inflammatory conditions in neurodegenerative diseases, a variety of immune regulatory and inflammatory mediators can be activated. Cytokines are key regulators of innate and adaptive immune responses. Although various types of cells including tissue infiltrating immune cells, neurons, microglia and astrocytes have been identified as sources of cytokines in the CNS, microglia appear to be a principal source of proinflammatory and immune regulatory cytokines.

IL-1 and TNF-α are two main proinflammatory cytokines produced by microglia during CNS inflammation. Pathogens or pathogen components such as LPS can immediately induce IL-1 and TNF-α both in cultured microglia and in LPS-injected rats (Appel *et al.*, 1995; Buttini and Boddeke, 1995). IL-1 and TNF-α have been shown to be involved in the development of CNS inflammation through the disruption of the BBB, the induction of adhesion molecules and chemokines from astrocytes and endothelial cells, which facilitate the infiltration of leukocytes into the CNS (Oh *et al.*, 1999; Sedgwick *et al.*, 2000). In addition, recent studies employing TNF-α over-expressing mice demonstrate that TNF-α may directly initiate CNS tissue destruction as well as maintain autoimmune inflammation (Taupin *et al.*, 1997; Akassoglou *et al.*, 1998).

Although the studies in the past have focused on microglial production of proinflammatory cytokines, recently, a large body of evidence has supported the notion that microglia also produce cytokines with anti-inflammatory activity, such as TGF-β, IL-10, and IL-1 receptor antagonist (IL-1Ra). IL-1Ra counteracts the biological effect of IL-1 by binding to the IL-1 receptor without initiating signal transduction. Both TGF-β and IL-10 inhibit microglial activation through their ability to inhibit antigen presentation and proinflammatory cytokines, chemokines and reactive oxygen intermediates (Frei *et al.*, 1994; Aloisi *et al.*, 1999a; O'Keefe *et al.*, 1999). Additionally, microglia can release reactive oxygen intermediates that may cause neuronal damage, express Fas and FasL, which mediate apoptosis of immune cells by microglia and also express growth factors that support neuronal survival.

In summary, a number of studies have demon-

strated that microglia play an important role in inflammatory conditions in the CNS as a principal source of a variety of inflammatory and immune regulatory mediators.

Molecules involved in microglial activation and signal transduction pathways

Several molecules, including LPS, IFN-γ, Aβ, CD40L, chemokines, neurotransmitters and gangliosides, and several proteases such as thrombin, tissue plasminogen activator (tPA) and matrix metalloproteinase-3 (MMP-3) are found to be involved in microglial activation. Some of them are from neurons and others are from pathogens, immune cells or microglia (abd-el-Basset and Fedoroff, 1995; Tsirka, 1997; Suzumura *et al.*, 1998; Tan *et al.*, 1999a; Aloisi *et al.*, 1999b; Moller *et al.*, 2000; Kim *et al.*, 2005).

LPS, an endotoxin from the gram-negative bacterial cell wall, is a potent immunostimulant (abd-el-Basset and Fedoroff, 1995). Although it is widely used in brain inflammatory studies, its action mechanism and signal transduction pathways are not fully elucidated. Several receptors for LPS were identified from a variety of studies. CD14, a GPI-anchored glycoprotein expressed in monocytes/ macrophages, reacts with LPS (Wright *et al.*, 1990). LPS forms a complex with LPS-binding protein (LBP) in serum (Wright *et al.*, 1990). Apart from CD14, CD11c/CD18, a member of the leukocyte integrin family, also activates cells in response to LPS (Ingalls *et al.*, 1998). Recently, toll-like receptor 4 (Tlr4) was identified by positional cloning as the LPS signal transducer from the Lps locus, mutations of which abolish responses to LPS (Hoshino *et al.*, 1999). A variety of intracellular signaling molecules, such as protein tyrosine kinases, mitogen-activated protein kinases, protein kinase C, small G proteins, and ceramide-activated protein kinase are involved in LPS-mediated monocytes/ macrophage activation (Sweet and Hume, 1996). Through different signal transduction pathways, LPS activates transcription factors including NF-κB, NF-IL6, C/EBP and Fos/ Jun families, and induces iNOS, TNF-α, IL-1β, IL-6, IL-12p40, TGF-β and other cytokine genes (Sweet and Hume, 1996).

IFN-γ, released from activated Th1 and NK cells, activates macrophages/microglia to increase expression of MHC class I and class II. With LPS, it synergistically induces IL-12 production from microglia (Aloisi *et al.*, 1997; Suzumura *et al.*, 1998). IFN-γ-mediated activation involves the JAK-STAT pathway. Briefly, IFN-γ stimulates the activation of receptor associated Jak1 and Jak2. This leads to the phosphorylation of a single receptor tyrosine residue, which is then recognized by the SH2 domain of Stat. It causes Stat phosphorylation followed by homodimerization, translocation into nucleus and induction of GAS (gamma-activation site) driven target genes (Schindler, 1999).

Chemokines are small proteins (8 to 10 kDa) that induce chemotaxis, tissue extravasation and functional modulation of a wide variety of leukocytes during inflammation (Taub, 1996). More than 40 distinct members are divided into 4 families typified by conservation of cysteine residues in the N-terminal sequence (Lusti-Narasimhan *et al.*, 1996). Chemokines mediate their effects via G protein-coupled receptors of the seven transmembrane domain (Horuk, 1994). A number of chemokines are expressed in the CNS. They are related to a number of diseases of the CNS including stroke, AIDS dementia, MS and AD (Mennicken *et al.*, 1999). Fractalkine/neurotactin is a unique member of CX3C chemokine family which was discovered in 1997 (Bazan *et al.*, 1997). In the CNS, several populations of neurons express fractalkine mRNA constitutively that is not affected by stimuli such as cytokines, LPS and toxic stimuli (Aβ, glucose deprivation or glutamate) (Maciejewski-Lenoir *et al.*, 1999). Membrane-bound fractalkine protein levels were decreased after excitotoxic glutamate stimuli (Chapman *et al.*, 2000). Its receptor, CX3CR-1 is expressed at high levels in microglia (Nishiyori *et al.*, 1998). Through its receptor, fractalkine induces intracellular Ca^{2+} mobilization, ERK activation and PI3-K-mediated PKB activation in microglia. It might be involved in regeneration of motor neurons after peripheral axotomy in facial motor neuron injury models (Harrison *et al.*, 1998). Recent study demonstrated that CX3CR-1 deficiency dysregulates microglial responses, resulting in neurotoxicity (Cardona *et al.*, 2006). Study showed that in the absence of CX3CR-1/fractalkine signaling, microglial responses to LPS and neurotoxic stimuli was altered. *Cx3cr1*$^{-/-}$ mouse demonstrated more dopaminergic neuronal loss in MPTP-treated Parkinson's disease model and in a model of genetic motor neuron disease.

CD40 is a 45-50 kDa transmembrane protein, which is a member of the TNFR superfamily (Vogel and Noelle, 1998). It has been shown that CD40 is constitutively expressed at low levels on microglia, and binding of microglial CD40 by CD40 ligand (CD40L) leads to marked TNF-α secretion, which is neurotoxic at such levels (Aloisi *et al.*, 1999b; Tan *et al.*, 1999a). Activation of ERK1/2 is involved in CD40-CD40L mediated microglial activation (Tan *et al.*, 2000a). Interestingly, stimulation with Aβ peptides and CD40L results in increased CD40 ex-

pression on microglia followed by TNF-α secretion (Tan *et al.*, 1999b). Recently, it has been demonstrated that CD45 suppresses CD40L-induced microglial activation via negative regulation of the Src/ERK1/2 cascade (Tan *et al.*, 2000b).

Amyloid-β peptide (Aβ) is the principal component of the extracellular deposits in AD (Selkoe, 1989). Aβ promotes neurite outgrowth, generates reactive oxygen intermediates, induces cytotoxic cellular oxidative stress, and microglial activation (Koo *et al.*, 1993; Behl, 1997; Sasaki *et al.*, 1997). Although the mechanism by which Aβ peptides cause enhanced expression of proinflammatory cytokines from microglia, is not fully understood there is evidence that Aβ may interact with cell-surface receptors, including receptors for advanced glycosylated endproducts (RAGE) and scavenger receptors (El Khoury *et al.*, 1996; Yan *et al.*, 1996). Additionally, calcium-, protein kinase C, and protein tyrosine kinase-dependent second messenger pathways have been postulated in Aβ receptor-mediated signal transduction (Lorton, 1997; Combs *et al.*, 1999). Aβ peptide activates microglia through these signal transduction pathways to induce the secretion of neurotoxic substances including TNF-α and IL-1β (Mrak and Griffin, 2001; Smits *et al.,* 2001). It may enhance neuroinflammation in AD brain.

Gangliosides, sialic acid-containing glycosphingolipids, have also been reported as microglial activators (Pyo *et al.*, 1999). Gangliosides exist in mammalian cell membranes and are particularly rich in the neuronal cell membrane. Gangliosides induce production of nitric oxide, TNF-α and cyclooxygenase-2 (COX-2) in microglia by activation of MAPKs (Pyo *et al.*, 1999). Recent studies show that signals are released from neurons when they start to die. Upon potassium deprivation, cerebellar granule cells release signal molecules that can activate microglia (Tanaka *et al.*, 1998). Supernatant from serum-deprived immortalized motor neurons can also activate microglia and induce release of NO that causes neuronal death (He and Strong, 2000). These signals from dying neurons may be potent candidates for microglial activation.

Thrombin-mediated microglial activation has been reported (Moller *et al.*, 2000; Suo *et al.*, 2002). Thrombin is generated from the precursor prothrombin that is endogenously expressed in human, mouse, and rat brain, including dopaminergic neurons in the SN (Dihanich *et al.*, 1991; Soifer *et al.,* 1994; Weinstein *et al.*, 1995). Thrombin-induced microglial activation involves protease-activated receptor-1 (PAR-1) (Suo *et al.*, 2002). Recent studies demonstrated that direct injection of thrombin into various brain parenchyma including hippocampus and substantia nigra results in induction of iNOS, COX-2 and NADPH oxidase-mediated superoxide generation from microglial and subsequent neuronal degeneration (Choi *et al.*, 2003; 2005).

We have recently identified matrix metalloproteinase-3 as a signaling molecule that is released from apoptotic cells and elicit microglial activation (Kim *et al.,* 2005). This will be discussed in detail later.

Roles of activated microglia in degenerative human brain disorders

The most characteristic feature of microglia is their rapid activation in response to pathological change in the CNS. They respond not only to changes in the brain parenchymal integrity but also to very small alterations in their microenvironment, such as imbalances in ion homeostasis that precede pathological changes (Gehrmann *et al.*, 1993). Although they have a critical role in host defense by removing invading microorganisms and neoplastic cells, or by secreting neurotrophic factors, microglia may aggravate the effects of inflammation and cause neuronal degeneration. Activated microglia at the site of inflammation change their morphology, express increased levels of MHC antigens and become phagocytic (Hayes *et al.*, 1987; 1988). They release inflammatory cytokines that amplify the inflammatory response by activating and recruiting other cells to the brain lesion. In addition, microglia can release potent neurotoxins, such as TNF-α and others, which may cause neuronal damage. There are several neurodegenerative diseases in which microglial activation and microglial function may play a more significant role in mediating the diseases than in protecting neurons. Among them are HIV infection of the CNS, MS, AD and PD (McGeer *et al.*, 1988; Raine, 1994; Dickson, 1997; Banati *et al.*, 1998).

HIV

Approximately 20% of HIV-infected individuals develop a neurological syndrome consisting of motor dysfunction, cognitive deterioration and coma in later stages. The most characteristic feature of HIV-infected brain is that virus is concentrated in microglia and macrophages, with a very limited infection of astrocytes, endothelial cells, or neurons (Gabuzda *et al.*, 1986; Wiley *et al.*, 1986). Recent studies have suggested the model in which HIV entry into the CNS is mediated by circulating lymphocytes or monocytes, which in turn transmit virus to perivascular macrophages and microglia (Lane *et al.*, 1996). The virus may replicate enough in infected microglia and macrophages to maintain a cycle of new infection. Infected microglia secrete several candidate neuro-

toxins, such as viral proteins (gp120, tat and nef), cytokines, chemokines, arachidonic acid, and nitric oxide, to induce neuronal death or injury.

MS

Pathological studies on MS brain demonstrate the important role of macrophages and microglia in MS demyelination. Experimental allergic encephalomyelitis (EAE), a disease induced in rats or mice by immunization against components of myelin, has been widely used as an MS animal model. The earliest lesion in EAE is characterized by T-cell infiltration, which is eventually followed by recruitment of inflammatory cells, including activated macrophages and microglia. These cells secrete cytokines and other potent neurotoxins and result in neurological damage. Microglia may have a role in the induction of the initial inflammatory reaction by antigen presenting. T-cell recruitment by microglial antigen-presenting activity in turn enhances microglial activation and they aggravate the damage of oligodendrocytes, myelin and axon by releasing neurotoxins (Raine, 1994).

AD

AD is a degenerative disorder characterized pathologically by β amyloid-containing extracellular plaques and intraneuronal neurofibrillary tangles (NFTs) (Goedert *et al.*, 1991). A large number of microglia form clusters around senile plaques. Initial detection of activated microglia in AD tissue used antibodies to the class II major MHC protein HLA-DR (McGeer *et al.*, 1987). They also demonstrated that HLA-DR protein was more abundantly expressed on microglia that are closely associated with areas of degenerative lesion in tissue from AD patients. The finding of microglial clustering around senile plaques demonstrated that microglia are trying to phagocytose and remove toxic Aβ plaques. However, microglia are known to produce many cytotoxic agents including proteolytic enzymes, cytokines, excitatory amino acids, quinolinic acid, complement proteins, reactive oxygen intermediates, and nitric oxide (Chao *et al.*, 1992; Cassarino *et al.*, 1997; McGuire *et al.*, 2001; Liu *et al.*, 2002). In addition, Aβ can not only recruit and induce phagocytic activity of microglia, but also synthetic Aβ also can induce the secretion of these cytotoxic agents (Meda *et al.*, 1995; Sasaki *et al.*, 1997). Therefore, it is conceivable that microglia, rather than being protective, may aggravate neurotoxicity in AD brain.

PD

PD is a common neurodegenerative disorder characterized by the degeneration of dopaminergic neurons in the substantia nigra pars compacta (SNpc) and the consequent loss of their projecting fibers in the striatum (Olanow and Tatton, 1999). The presence of oxidative stress and inflammatory activity is one of the significant pathological features of PD (Dexter *et al.*, 1994; Hirsch *et al.*, 1998). It has been shown that the levels of cytokines such as TNF-α, IL-1β and IFN-γ are elevated by 7- to 15-fold in the SN of PD patients (Mogi *et al.*, 1994; Hirsch *et al.*, 1998). Since microglia are a principal source of these cytokines, the data support microglial involvement in the pathogenesis of PD. Activated microglia-mediated dopaminergic neuronal degeneration has been demonstrated in recent studies using animal models. Gao *et al.,* (2002) showed that microglia play a pivotal role in rotenone-induced dopaminergic neuronal degeneration. Wu *et al.,* (2002) demonstrated that the inhibition of microglial activation prevents dopaminergic neuronal loss in MPTP-treated mice. Microglial role in PD patogenesis will be discussed in depth (Figure 3).

Pathological dynamics of activated microglia in pathogenesis of PD

Activated microglia have been implicated in the pathogenesis and progress of PD. Characteristic pathological features of the PD brain are a selective and progressive loss of dopamine neurons of the substantia nigra (SN) and focal accumulation of activated microglia in the SN (McGeer *et al.*, 1988; Forno, 1996; Banati *et al.*, 1998; Knott *et al.*, 2000; Mirza *et al.*, 2000). Activation of microglia also has been identified in the SN and/or striatum of the parkinsonian animal models, such as 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced parkinsonian brain (O'Callaghan *et al.*, 1990; Francis *et al.*, 1995; Czlonkowska *et al.*, 1996; Kohutnicka *et al.*, 1998; Kurkowska-Jastrzebska *et al.*, 1999) or medial forebrain bundle (MFB), which transmits axons from the nigral dopamine neurons to the striatum, axotomized rat brain (Brecknell *et al.*, 1995; Revuelta *et al.*, 1999; Sugama *et al.*, 2003). Our recent study elucidated the neuropathological dynamics of activated microglia and their pathophysiological role during delayed neuronal loss in the SN (Cho *et al.*, 2006). In this study, we examined immunophenotypic and morphological changes of activated microglia, as well as their temporal and spatial relationship with degenerating dopamine neurons after medial forebrain bundle (MFB) axotomy in the rat brain. Activated microglia appeared in the MFB and SN as early as 1-3 days post-lesion (dpl), when there was no apparent SN dopamine

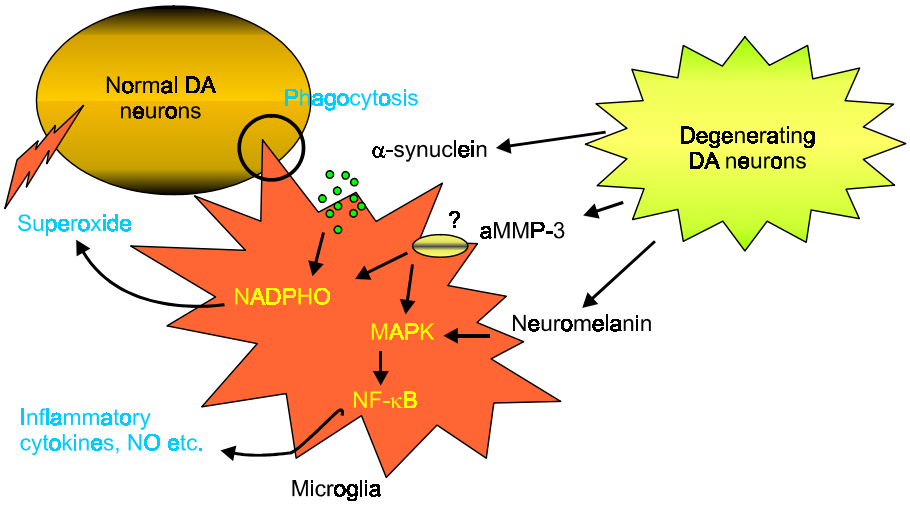

Figure 3. Microglia-mediated self-perpetuating dopamine (DA) neuronal degeneration model of PD. Various stimuli (either internal or external) initiating dopamine degeneration result in microglial activation through stimulatory signaling molecules such as aMMP-3 (active form of MMP-3), α-synuclein and neuromelanin leakage. aMMP-3 induces NADPH oxidase (NADPHO)-derived superoxide release and NF-κB-mediated proinflammatory cytokine release through yet undefined pathway, probably protease-activity related receptor. Neuromelanin elicits microglial proinflammatory cytokine and NO release through NF-κB pathway. It has not been investigated yet whether it causes NADPHO-derived superoxide generation. α-synuclein, especially aggregated form causes NADPHO-mediated superoxide release via phagocytosis-dependent pathway. Activated microglia cause dopamine neuronal degeneration either by superoxide, NO and other proinflammatory cytokines or by direct phagocytosis against normal neurons. This self-propelling degeneration cycles sustain chronic inflammatory condition and eventually induce progressive degeneration.

neuronal degeneration. Thereafter, a great number of activated microglia selectively adhered to degenerating axons, dendrites and dopamine neuronal somas of the SN. Thereafter, significant loss of these fibers and nigral dopamine neurons was observed. Although phenotypical activation of microglia was most pronounced between 14-28 dpl and gradually subsided, phagocytic microglia persisted until 70 dpl. All phagocytic microglia sticking to dopamine neurons showed activated but ramified form with enlarged somas and thickened processes. They were recruited to the SNc from cranial, dorsal and ventral aspects along various structures and finally stuck to dopamine neurons of the SNc. Characteristic rod-shaped microglia in the white matter were thought to migrate a long distance (Cho *et al.*, 2006). Our results strongly suggests that neurons undergoing delayed neurodegeneration may be phagocytosed by numerous phagocytic, ramified microglia at various sites where specific surface signals are exposed or diffusible molecules are released. In addition, our earlier results (Sugama *et al.*, 2003) showed that activated microglia were phargocytosing dopamine neuronal fibers at early stage of neuronal degeneration, suggesting that microglial phagocytosis of degenerating neurons is early occurring in neuronal degeneration, starting phagocytosis at the extending fibers, such as neurite extended to the SN reticulata. These results imply that degenerating neurons may release microglia-activating signals much earlier stages of neuronal degeneration, and activated microglia start ingesting the nerve fibers piece-by-piece (Sugama *et al.*, 2003). Our recent unpublished data (Cho *et al.,* 2006) also showed that ramified microglia, probably after digestion of pieces of degenerating neurons, increased their sizes, which are nearly equivalent to the size of neuronal cell bodies (approximately 50 μm), and apposed onto neighboring, bystander, non-degenerating neurons. The electron micrographic analysis of these microglia suggested that large, ramified microglia phagocytose bystander dopamine neurons, suggesting that activated microglial participation in the progressive degeneration of dopamine neurons (Cho *et al.*, unpublished).

Matrix metalloproteinase-3 (MMP-3)

Although a number of studies have demonstrated microglial activation and its involvement in progressive neuronal degeneration upon dopamine neuronal damage, molecular mechanism underlying microglial

activation and signals responsible for chronic microglial inflammatory response are still disputed. Our previous study identified matrix metalloproteinase-3 (MMP-3), a proteinase known to degrade extracellular matrix (ECM) component, as a candidate which intermediates neuronal apoptosis and microglial activation. In this study, we demonstrated that catalytically active from of MMP-3 (actMMP-3) is released from apoptotic PC12 cells grown in serum-deprived medium. actMMP-3 release occurred as early as 2 hr after serum deprivation, even before cellular morphological change. actMMP-3 led to production of microglial inflammatory cytokines such as TNF-α through ERK-NFκB signal transduction pathway, which in turn exacerbated neural cell degeneration (Kim *et al.*, 2005). In our current work, MMP-3 expression and release were also shown in primary mesencephalic culture treated with 1-methyl-4-phenylpyridinium (MPP+), a toxic metabolite of MPTP, a specific dopamine toxin. MPP+ treatment resulted in dopamine cell-specific expression of MMP-3 and dose dependent increase of actMMP-3 in culture medium. The released actMMP-3 as well as catalytically active recombinant MMP-3 (cMMP-3) led to microglial activation and superoxide generation in microglia and enhanced dopamine cell death. cMMP-3 caused dopamine cell death in mesencephalic neuron-glia mixed culture of wild-type mice but this was attenuated in the culture of NADPH oxidase (NADPHO) subunit null mice (*gp91$^{phox-/-}$*), suggesting that NADPHO mediated the cMMP-3-induced microglial production of superoxide and dopamine cell death (Kim *et al.*, in press). This is critical finding to understand microgliosis in PD, as previous studies have identified that NADPHO-mediated microglial superoxide production is a mandatory microglial component to contribute MPP+ and MPTP induced dopamine toxicity both *in vivo* and *in vitro* (Wu *et al.*, 2002; Gao *et al.*, 2003). Furthermore, in the MPTP-injected animal model of PD, nigrostriatal dopamine neuronal degeneration, microglial activation and superoxide generation were largely attenuated in *MMP-3*-/- mice. Together, these data indicate that actMMP-3 released from stressed dopamine neurons is responsible for microglial activation and generation of NADPHO-derived superoxide, and eventually propagates nigrostriatal dopamine neuronal degeneration.

α-Synuclein

Cytoplasmic accumulation of fibrillar α-synuclein in Lewy bodies (Spillantini *et al.*, 1997), the pathological hallmark of PD, may play a pivotal role in the onset and progression of Parkinson's as well as other neurodegenerative diseases. Although pathophysiological role of this protein in the dopamine degeneration is still not clear, a wealth of evidence suggest that abnormal accumulation and aggregation of wild type or mutants (A53T, A30P and E46K) α-synuclein may directly cause dopamine neuronal death. α-synuclein is generally considered an intracellular protein, because of the lack of signal sequence and its abundant presence in the cytosol. However, recent studies proved that small portion of newly synthesized α-synuclein is secreted from cells. Interestingly, the intravesicular α-synuclein has increased aggregation tendency, secretion of both monomeric and aggregated α-synuclein is elevated in response to proteasomal and mitochondrial dysfunction (Lee *et al.*, 2005). Evidence of α-synuclein release from cells is the presence of full length α-synuclein in cerebrospinal fluid from Parkinson's disease and normal subjects (Borghi *et al.*, 2000), and in human plasma (El-Agnaf *et al.*, 2003). Several studies demonstrated that extracellular Lewy bodies and α-synuclein-immunoreactive nigral aggregates are often surrounded by microglial or inflammatory mediator such as complements (McGeer *et al.*, 1988; Yamada *et al.*, 1992). Recent interesting study by Zhang *et al.* implies that microglia can enhance α-synuclein-induced dopamine toxicity (2005). They also showed that α-synuclein aggregates fail to induce dopamine neurotoxicity in microglial-depleted mesencephalic culture at low concentration. α-synuclein aggregates induced intracellular ROS in microglia and superoxide release. Since cytochalasin D, an inhibitor of phagocytosis, inhibited α-synuclein-mediated ROS generation in microglia, implying that phagocytosis is a critical component of the α-synuclein aggregates-mediated microglial activation.

Neuromelanin

Neuromelanin is the dark insoluble macromolecule that confers the black (substantia nigra) or grey (locus coeruleus) color to monoaminergic basal ganglia. Neuromelanin accumulates in normal healthy human SN with age. Normally, neuromelanin is considered to play a protective role intracellularly by binding toxic metabolite produced in SN cells such as dopamine and metals (D'Amato *et al.*, 1986; Lindquist *et al.*, 1988; Zecca *et al.*, 1994) and serve as an antioxidant (Fornstedt *et al.*, 1989; Wilczok *et al.*, 1999). However, it has been also suggested that neuromelanin can be potentially toxic to dopamine neurons, as excess neuromelanin directly inhibits proteasomal function (Shamoto-Nagai *et al.*, 2004; Shamoto-Nagai *et al.*, 2006). Several studies showed that neuromelanin also exists outside dopamine neurons. Extraneuronal melanin was found in pa-

tients suffering from juvenile (Ishikawa and Takahashi, 1998), idiopathic, and MPTP-induced parkinsonism (Langston *et al.*, 1999). In an autopsy study of a patient with MPTP-induced parkinsonism, extraneuronal melanin was found in close vicinity to activated microglial cells even 12 years after exposure to the neurotoxin. This suggests that neuromelanin interacts with microglia. By adding neuromelanin extracted from PD brain to microglia culture, Wilm *et al.* showed that neuromelanin has chemotactic effects, and induces microglial proinflammatory cytokines such as TNF-α, IL-6 and NO through p38 MAPK-NFκB signal transduction pathways. Those molecules have been implicated as factors involved in PD pathogenesis (Wilms *et al.*, 2003).

Molecules such as MMP-3, α-synuclein and neuromelanin that can be released from damaged dopamine neurons might form self-perpetuating vicious cycles of neuronal degeneration by augmenting microglial activation and eventually lead to chronic inflammation.

Conclusion

Activation of microglia is implicated in the pathogenesis of various neurodegenerative diseases which are characterized by long-term progressive nature of degeneration. A number of stimuli might trigger neuronal death under various circumstances and microglia might be activated either by various signals released from dying neurons or by direct contact to damaged neurons. By releasing toxic molecules including superoxide, or by actively phagocytosing by-stander neighboring cells, activated microglia may form vicious self-perpetuating neuronal degeneration cycle. Thus, intervention of microglial activation process will become a promising therapeutic target for the treatment of a number of neurodegenerative conditions (Figure 3).

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