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Microglial Functions and Proteases

Hiroshi Nakanishi*

Laboratory of Oral Aging Science, Faculty of Dental Sciences, Kyushu University, Fukuoka 812-8582, Japan

Abstract

There is accumulating evidence that intracellular and extracellular proteases of microglia contribute to various events in the central nervous system (CNS) through both nonspecific and limited proteolysis. Cathepsin E and cathepsin S, endosomal/lysosomal proteases, have been shown to play important roles in the major histocompatibility complex (MHC) class II-mediated antigen presentation of microglia by processing of exogenous antigens and degradation of the invariant chain associated with MHC class II molecules, respectively. Some members of cathepsins are also involved in neuronal death after secreted from microglia and clearance of phagocytosed amyloid-β peptides. Tissue-type plasminogen activator, a serine protease, secreted from microglia participates in neuronal death, enhancement of *N*-methyl-D-aspartate receptor-mediated neuronal responses, and activation of microglia via either proteolytic or nonproteolytic activity. Calpain, a calcium-dependent cysteine protease, has been shown to play a pivotal role in the pathogenesis of multiple sclerosis by degrading myelin proteins extracellulary. Furthermore, matrix metalloproteases secreted from microglia also receive great attention as mediators of inflammation and tissue degradation through processing of pro-inflammatory cytokines and damage to the blood–brain barrier. The growing knowledge about proteolytic events mediated by microglial proteases will not only contribute to better understanding of microglial functions in the CNS but also may aid in the development of protease inhibitors as novel neuroprotective agents.

Index Entries: Microglia; protease; antigen presentation; neuronal death; inflammation.

Introduction

Microglia are distributed in the central nervous system (CNS) and comprise up to 20% of the total glial cell population. In the normal CNS, microglia are present as ramified cells which have small cell bodies with numerous branching processes. In response to neuronal injury, ramified microglia rapidly transform into activated states. As an intermediate form, microglia have large-cell bodies with several thicker processes. If neurons are severely injured, microglia further transform into

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*Author to whom all correspondence and reprint requests should be addressed. E-mail: nakan@dent.kyushu-u.ac.jp

phagocytic microglia. Although activated microglia are known to have both beneficial as well as harmful effects, little is known about the molecular basis which determines the outcome of microglial activation in the CNS.

There is growing evidence that intracellular and extracellular proteolytic systems are closely associated with functions of microglia. Considering that proteases can irreversibly cleavage peptide bonds, functions of proteases can be classified into two major categories. One is their "disintegrating action" by which physiological functions of substrates are inactivated after cleavage. The other is their "modulatory action" by which substrates are activated after cleavage. Therefore, microglial proteases are also considered to exert both beneficial and harmful effects. For better understanding of microglial functions, it is important to elucidate the key substrates for each microglial protease. Here, the proteolytic systems are reviewed, which are directly or indirectly associated with microglial functions in the CNS.

Cathepsins

Cathepsins B, D, and L are ubiquitously present in the endosomal/lysosomal system of various tissue cells. On the other hand, cathepsins E and S are expressed largely in the endosomal/lysosomal system of mononuclear phagocytic lineage, suggesting their tissue-specific functions.

Antigen Presentation

Some members of cathepsins are responsible for two proteolytic pathways required for the major histocompatibility complex (MHC) class II-mediated antigen presentation: a degradation of invariant chain (Ii) associated with MHC class II and endocytosed exogenous antigens. Although cysteine proteases are critical for the terminal step of Ii breakdown, their exact role varies among different antigen-presenting cells (Villadangos et al., 1999; Rieses and Chapman, 2000). In B cells and dendritic cells, cathepsin S exclusively mediates the degradation of Ii to class II-associated Ii peptide (CLIP) during maturation of MHC class II molecules. For this purpose, thymic cortical cells and macrophages use cathepsin L and cathepsin F, respectively. On the other hand, aspartic protease inhibitors such as pepstatin A prevent antigen processing and Ii degradation (Maric et al., 1994; Zhang et al., 2000). In B cells, expression level of cathepsin E was upregulated upon cellular activation and specific inhibitor of cathepsin E blocked the presentation of ovalbumin (OVA) (Bennett et al., 1992; Sealy et al., 1996). Furthermore, it has been reported that cathepsin D generated antigenic peptides from OVA or hen egg lysozyme that could be presented to T cells (Diment, 1990; Rodriguez and Diment, 1992; van Noort and Jacobs, 1994). More recently, however, the experiments conducted with splenocytes and macrophages prepared from cathepsin D-deficient mice have demonstrated that cathepsin D is dispensable for degradation of Ii and processing of a number of exogenous antigens (Villadangos et al., 1997; Deussing et al., 1998).

In the CNS, microglia are known to interact with invaded $CD4^+$ T-helper cells. It has been suggested that microglia use cathepsin S or cathepsin L for the degradation of Ii to CLIP because microglia express cathepsin S and cathepsin L, but not cathepsin F (Gresser et al., 2001; Santambrogio et al., 2001). However, it is more likely that microglia utilize cathepsin S and cathepsin B but not cathepsin L in the degradation of Ii to CLIP (Nishioku et al., 2002). In interferon-γ (IFN-γ)-treated primary cultured murine microglia, pepstatin A inhibited interleukin-2 (IL-2) production from OVA (266–281)-specific T-helper cell hybridomas upon stimulation with native OVA but not with OVA (266–281) peptide (Fig. 1A). On the other hand, the degradation of Ii chain was not inhibited by pepstatin A. Furthermore, microglia isolated from cathepsin D-deficient mice retained the ability for antigen presentation (Fig. 1B) (Nishioku et al., 2002). In mammalian cells, cathepsin D and cathepsin E are only known for pepstatin A-sensitive endoso-

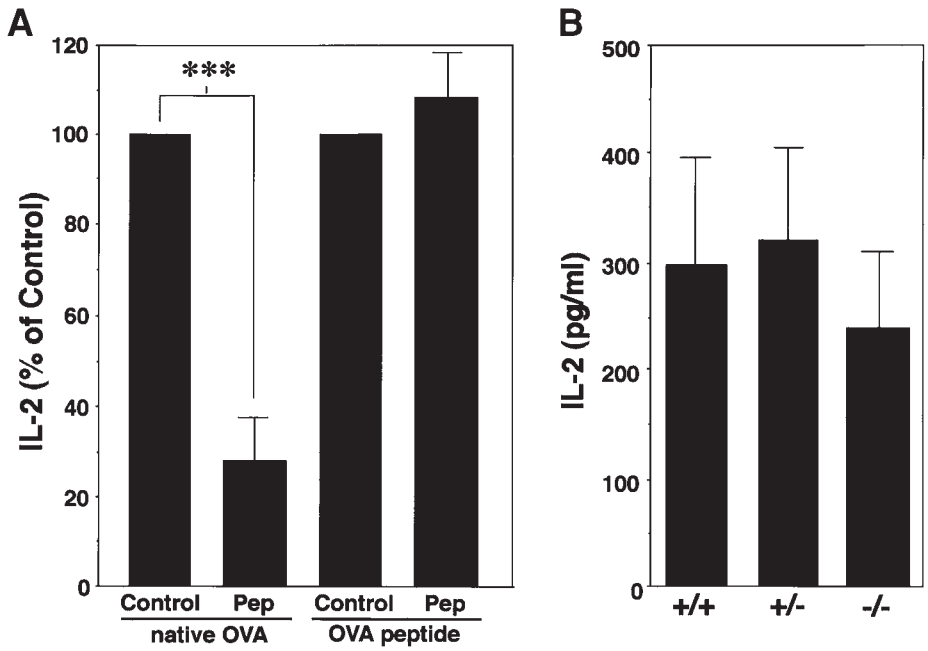

Fig. 1. Involvement of cathepsin E in OVA processing in primary cultured murine microglia. **(A)** Effects of pepstatin A on antigen presentation of IFN-γ-treated microglia. Pepstatin A (Pep, 10 μM) was applied to culture medium 24 h before adding OVA-(266–281)-specific T helper cell hybridomas and native OVA or OVA(266–281) peptide. T helper cell-derived IL-2 was measured by ELISA. Each column and bar represents mean and SD of three experiments, respectively. Each value is expressed as a percentage of the amount to normalize the values with respect to the amount of IL-2 in the absence of inhibitor (control). ***$p < 0.001$ vs control (Student's *t*-test). **(B)** Effects of cathepsin D-deficiency on antigen presentation of microglia. OVA(266–281)-specific T helper cell hybridomas were cultivated together with IFN-γ-treated microglia prepared from cathepsin D-deficient mice (–/–), as well as their wild (+/+)- and hetero (+/–)-type littermates in the presence of native OVA. After 24 h, supernatant from triplicate cultures were harvested and T helper cell-derived IL-2 was measured by ELISA. Each column and bar represents mean and SD of three experiments, respectively.

mal/lysosomal aspartic proteases. Importantly, cathepsin E was found to be localized mainly in the endosomal structures of microglia (Sastradipura et al., 1998). In fact, the high pH of endosomes compaired with lysosomes favors limited proteolysis of endocytosed exogenous antigens. On the basis of these observations, it is conceivable that cathepsin E is responsible for the generation of antigenic peptides in microglia. Although the precise implication of exogenous antigen presentation in the CNS is not fully understood, inflammatory cytokines secreted from activated helper T cells and microglia activated through their interaction may contribute to tissue damage and repair during autoimmune disease, viral infections, and chronic inflammatory disease. Cathepsins and the two proteolytic pathways required for MHC class II-mediated exogenous antigen presentation in microglia are summarized in Fig. 2.

Degradation of Extracellular Matrix (ECM) Proteins and Neuronal Death

Besides functions in the endosomal/lysosomal system, there is evidence that cathepsin S and cathepsin B are also involved in extracel-

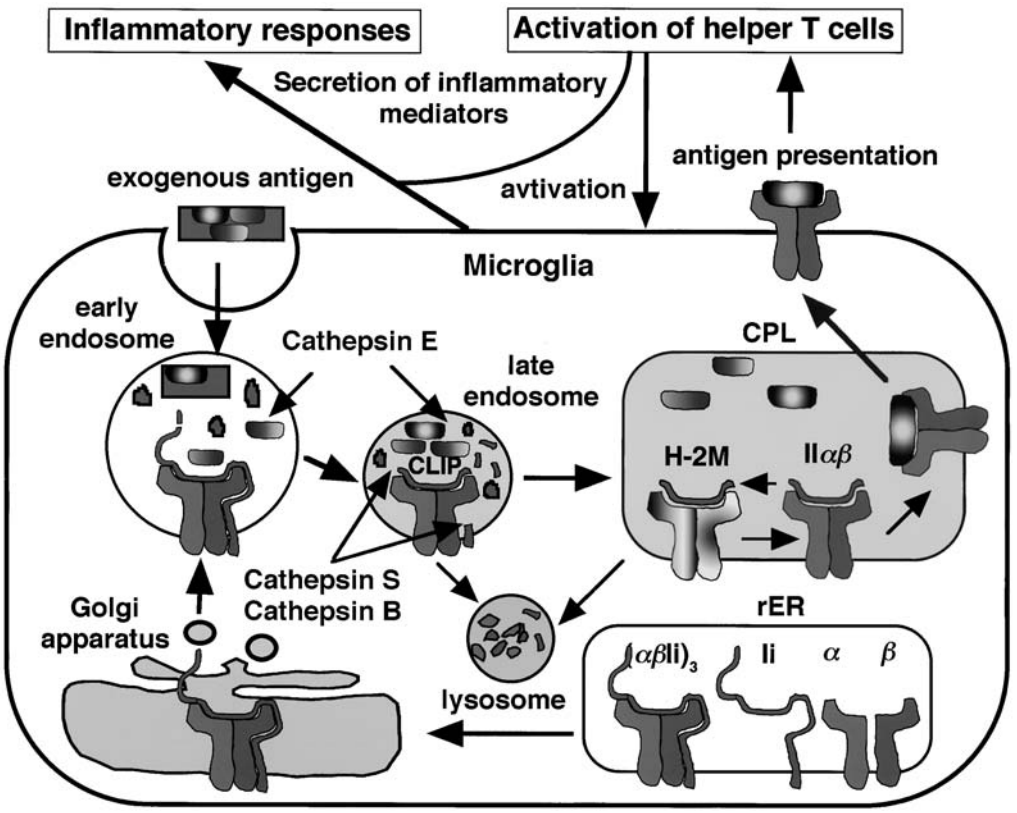

Fig. 2. Cathepsins involved in the MHC class II-mediated antigen presentation of microglia. Endocytosed exogenous antigens are degraded into antigenic peptides in endosomes in which cathepsin E plays an essential role. For maturation of the MHC class II molecules, Ii is degraded to generate CLIP by proteolytic activities of cathepsins S and B. The catalytic action of H-2M results in substitution of CLIP for antigenic peptides. MHC class II peptide complexes translocated on the cell surface display these antigenic peptides to interact with and activates helper T cells. The activated helper T cells may contribute to inflammatory responses in the CNS through secretion of inflammatory mediators. CPL, compartment for peptide loading; rER, rough endoplasmic reticulum.

luar proteolysis. In response to lipopolysaccharide (LPS), there is a substantial increase in cathepsin S activity secreted from both macrophages and microglia (Petanceska et al., 1996). This may suggest that cathepsin S plays a role in degenerative disorders because cathepsin S degrades components of ECM at neutral pH. Cathepsin B is also secreted from immortalized murine microglial cell line, BV-2 cells, as the heavy chain form in addition to the proform upon stimulation with LPS (Ryan et al., 1995). More recently, it has been demonstrated that secreted cathepsin B is a major causative factor of microglia-induced neuronal apoptosis (Kingham and Pocock, 2001). This observation is consistent with recent evidence that lysosomal proteases are involved in apoptosis. Cathepsin B has been implicated in the activation of the proinflammatory caspases 11 and 1 (Schotte et al., 1998; Vancompernolle et al., 1998). Furthermore, cathepsin B can cleave the Bcl-2 family member Bid (Stoka et al., 2001), which may lead to cytochrome c release from the mitochondria and subsequent caspase activation (Guicciardi et al., 2000).

Intracellular Degradation of Amyloid-β (Aβ) Peptides

It has been shown that cathepsin D is responsible for the intracellular clearance of Aβ peptides in human and rat brains (McDermott and Gibson, 1996; Hamazaki, 1996). Aβ peptides are taken up predominantly by microglia via class A scavenger receptors and class B scavenger receptor type I (Paresce et al., 1996; Husemann, 2001). Then Aβ peptides are accumulated and degraded in endosomal/lysosomal systems of microglia (Paresce et al., 1997). It has been also reported that the degradation of Aβ peptides in microglia is significantly inhibited by pepstatin A (Kakimura et al., 2002). These observations strongly suggest that phagocytosed Aβ peptides are mainly degraded by cathepsin D in microglia. It is also noteworthy that immunization with Aβ peptides has been demonstrated to reduce Aβ peptides in transgenic mice with Aβ plaques (Schenk et al., 1999). Anti-Aβ antibodies probably facilitate clearance of Aβ peptides by driving microglia to phagocytose Aβ peptides through Fc receptors. Thus the phagocytosis and subsequent degradation of Aβ peptides by microglia may play a pivotal role in a strategy for the immunotherapy of Alzheimer's disease.

Inflammation and Neuronal Death

We have recently reported that there was a prominent expression of inducible nitric oxide synthase (iNOS) in both morphological transformed microglia and peripheral macrophages in cathepsin D-deficient mice that have suffered from a massive intestinal necrosis and neuronal lysosomal storage associated with massive neurodegeneration (Koike et al., 2000; Nakanishi et al., 2001). Nitric oxide (NO) and the super oxide anion, which are generated in mitochondria, react rapidly to form a peroxinitrite anion. This, in turn, generates highly toxic hydroxyl radicals and hydrogen peroxide. Although NO is synthesized from L-arginine by NOS, iNOS is thought to be the isoform that produces the large quantities of NO that can result in tissue damage or death. To directly address the possible involvement of NO in tissue damage and neuronal death in cathepsin D-deficient mice, we examined effects of of L-N^G-nitro-arginine methylester (L-NAME), a potent competitive NOS inhibitor and S-methylisothiourea hemisulfate (SMT), an iNOS inhibitor. The chronic treatment of L-NAME or SMT significantly decreased the total number of terminal dUTP nick-end labeling (TUNEL)-positive cells counted in the thalamus of cathepsin D-deficient mice (Fig. 3) (Nakanishi et al., 2001). During the course of experiments, we have unexpectedly found that the chronic treatment of L-NAME or SMT markedly ameliorated a severe haemorrhagic–necrotic appearance of the small intestine and atrophic changes of the ileal mucosa of cathepsin D-deficient mice. Therefore, NO production via iNOS activity in microglia and peripheral macrophages contributes to secondary tissue damages such as neuronal death and intestinal necrosis, respectively. Our hypothesis for a mechanism underlying activation of microglia and subsequent neuronal death in the CNS caused by cathepsin D-deficiency is summarized in Fig. 4.

Plasminogen Activator, Plasmin, and Thrombin

Plasminogen activators (PA) are serine proteases that catalyze the conversion of plasminogen into plasmin which play an important role in fibrinolysis. Two distinct molecular forms, tissue-type (tPA) and urokinase-type (uPA), encoded by two different genes have been identified. On the other hand, thrombin is a serine protease that is critical to blood coagulation by catalyzing the conversion of fibrinogen into fibrin.

Induction of Neuronal Death

Flavin et al. (2000) have demonstrated that microglia induce apoptosis of primary cultured rat hippocampal neurons after activation

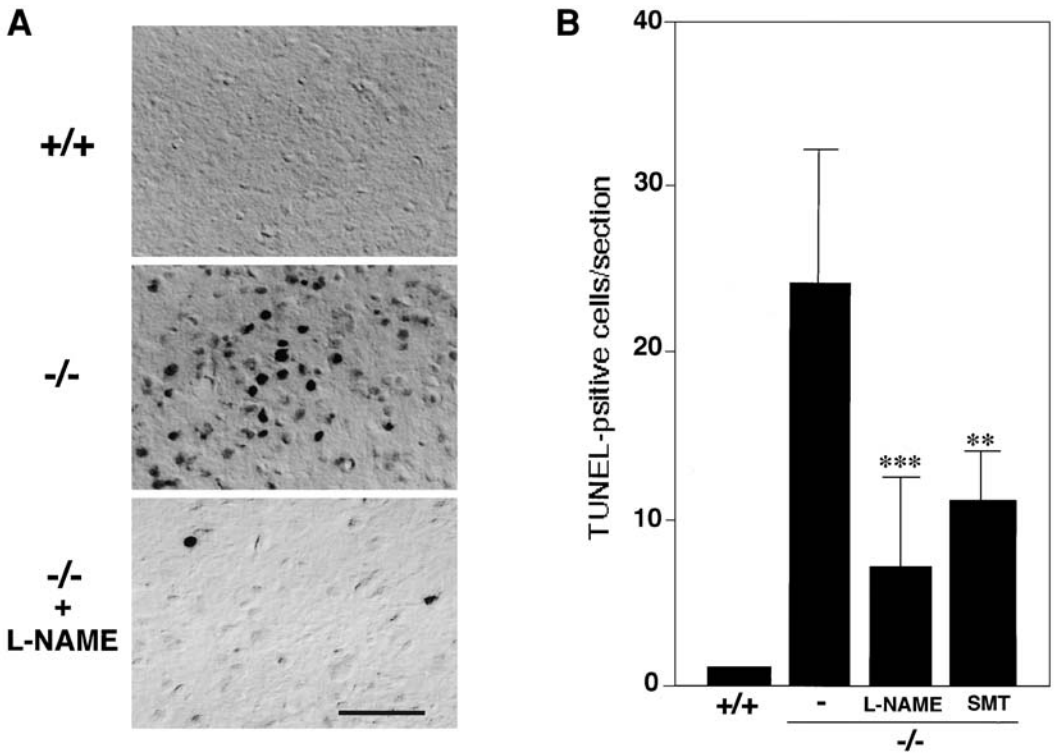

Fig. 3. Involvement of NO released from microglia in neuronal death. **(A)** TUNEL-stained sections in the thalamus of control littermates at P24 (+/+), cathepsin D-deficient mice at P24 (–/–), and cathepsin D-deficient mice treated with L-NAME at P24 (–/– + L-NAME). Scale bar = 100 μm. **(B)** Effects of chronic treatment with L-NAME or SMT on the number of TUNEL-positive cells in the thalamus of cathepsin D-deficient mice. The TUNEL-positive cells were counted in semisequential sagittal sections of the thalamus. Each column and bar represents mean and SD, respectively. $^{**}p < 0.01$, $^{***}p < 0.001$ vs control littermates (Student's *t*-test).

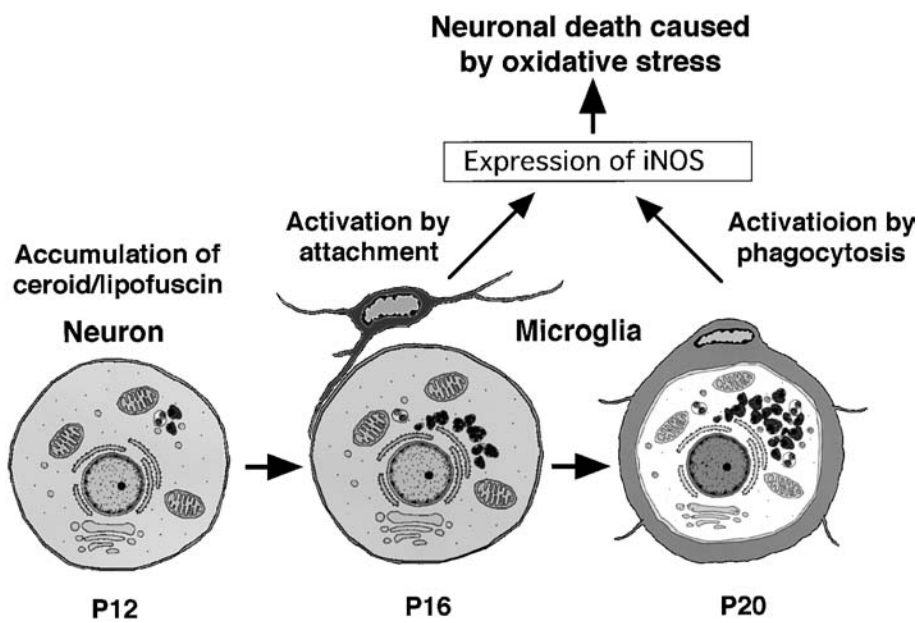

Fig. 4. Microglial activation and neuronal death in the CNS of cathepsin D-deficient mice. The deficiency for cathepsin causes lysosomal storage by formation and accumulation of ceroid/lipofuscin in neurons. By binding and/or phagocytosis of damaged storage neurons, microglia are activated to produce NO through iNOS. Microglial NO could induce neuronal death in adjacent neurons and an inflammatory response in the CNS.

with LPS. They have demonstrated that secreted tPA is a principal factor for the activated microglia-induced neuronal apoptosis because PA inhibitor-1 (PAI-1), tPA STOP, or co-incubation with anti-tPA antibody blocked it. More recently, however, Flavin and Zhao (2001) have reported that recombinant human tPA completely protected oxygen/glucose deprivation-induced cell death of primary cultured rat hippocampal neurons. This protective effect of tPA was abolished by anti-tPA antibody but not by PAI-1, indicating that the protective effect of tPA is not mediated by proteolytic activity.

Enhancement of NMDA Receptor-Mediated Responses

There is increasing evidence that serine proteases, which are well known for their roles in blood coagulation and fibrinolysis, also contribute to potentiation of *N*-methyl-D-aspartate (NMDA) receptor-mediated responses in the CNS (Gingrich et al., 2000; Nicole et al., 2001). Gingrich et al. (2000) have demonstrated that the thrombin-induced enhancement of NMDA receptor-mediated responses is caused by proteolytic activation of the potease-activated receptor-1 (PAR-1), but not by cleavage of NMDA receptors because a peptide agonist (SFLLRN) that activates PAR-1 can mimic the effects of thrombin. On the other hand, tPA has been shown to be necessary for late-phase LTP (L-LTP) in both the Schaffer collateral-CA1 and mossy fiber-CA3 pathways in the hippocampus by utilizing tPA-deficient mice (Huang et al., 1996) and specific inhibitors for tPA (Baranes et al., 1998). Furthermore, mice overexpression of tPA showed an enhanced LTP (Madani et al., 1999), and application of tPA enhanced L-LTP in rat hippocampal slices (Baranes et al., 1998). These observations strongly suggest that tPA plays a pivotal role in L-LTP. Plasminogen, a major substrate of tPA, is converted to the broad-spectrum protease plasmin, which can cleave ECM proteins such as laminin and fibronectin. However, plasmin-mediated extracellular proteolysis is unlikely as a causative factor of tPA-induced potentiation of L-LTP, because plasmin impaired the maintenance of LTP by degrading laminin (Nakagami et al., 2000). Zhuo et al. (2000) have demonstrated that the binding of tPA to its cell-surface receptor, the low-density lipoprotein receptor-related protein (LRP) activates cAMP-dependent protein kinase A (PKA) which plays a key role in L-LTP. More recently, Nicole et al. (2001) have demonstrated that tPA enhances NMDA receptor-mediated signaling by cleaving the NR1 subunit of the NMDA receptor. tPA was found to remove a fragment of approx 15–20 kDa from the amino terminus of the NR1 subunit. Taken together, it is conceivable that tPA contributes to L-LTP by both LRP-mediated activation of PKA and the cleavage of the NR1 subunit of the NMDA receptors.

Recently, we have found that microglia transferred onto cortical slice cultures potentiated NMDA receptor-mediated synaptic responses without affecting AMPA/kainate receptor-mediated responses. In cortical slice cultures transferred with primary cultured microglia, the subcortical white matter stimulation induced fast excitatory postsynaptic potentials (EPSPs) followed by plateau-like potentials which were never observed in control slice cultures under whole-cell patch recordings (Fig. 5A). Bath application of D, L-aminophosphovaleric acid (APV, 50 μ*M*), a competitive NMDA receptor antagonist, almost completely suppressed the plateau-like potentials without affecting the fast EPSPs (Fig. 5A). Furthermore, bath application of microglial conditioned-medium (MCM) also potentiated NMDA receptor-mediated EPSPs induced by the subcortical white matter stimulation in the acute cortical slices (Fig. 5B). This is consistent with previous reports demonstrating that microglia/macrophages induce NMDA receptor-mediated neuronal death by releasing a small heat-stable neurotoxic factor (<500 Da) (Giulian et al., 1993) or glutamate itself (Piani and Fontana, 1994). Plasminogen enhances glutamate-evoked increase in intracellular Ca^{2+} through modulation of activated NMDA receptors in hippocampal neurons (Inoue et al.,

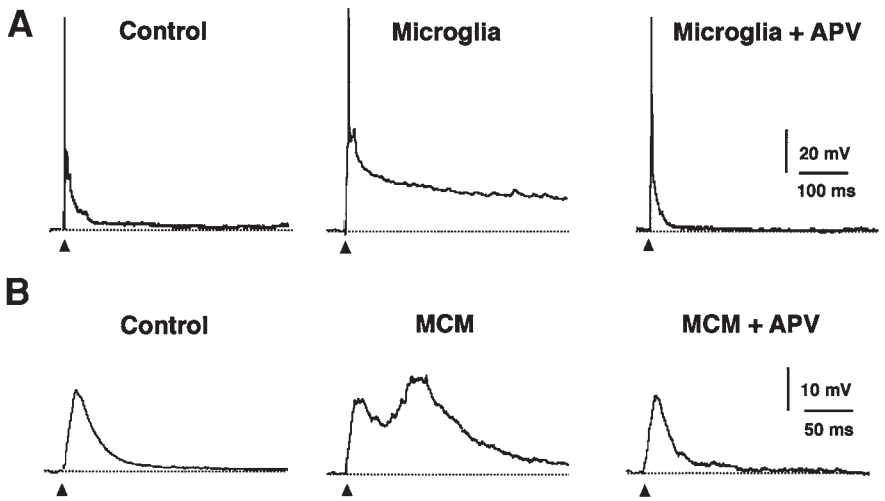

Fig. 5. Enhancement of NMDA-receptor-mediated responses by microglia or MCM. **(A)** Postsynaptic responses evoked by the subcortical white matter stimulation in the cortical slice culture (control), the cortical slice culture transferred with microglia at a cell density of 2×10^6 for 5 d (microglia), and the microglia-transferred cortical slice culture during bath application of APV (50 μM) (Microglia + APV). **(B)** Postsynaptic responses evoked bym the subcortical white matter stimulation in the acute cortical slice (control), MCM (10%, v/v)-treated acute cortical slice, and MCM-treated acute cortical slice during bath application of APV (50 μM) (MCM + APV).

1994). It is not likely that a small heat-stable neurotoxic factor or glutamate is involved in the MCM-induced potentiation of NMDA receptor-mediated EPSPs because the partially purified effector protein with a molecular weight of 70 kDa had a serine protease activity. The involvement of plasminogen can also be ruled out because MCM-induced potentiation of NMDA receptor-mediated EPSPs was significantly suppressed by anti-tPA antibody or PAI-1. The MCM-induced potentiation of NMDA receptor-mediated EPSPs may be due to a specific cleavage of NMDA receptors but not to a nonspecific proteolytic activity of tPA, because NMDA receptor antagonists or PAI-1 completely suppressed the effect of MCM. It is well known that neuronal injury caused by cerebral ischemia and trauma is accompanied by NMDA receptor-mediated hyperexcitability (Mittmann et al., 1994; Miyazaki et al., 1994). It has been also reported that tPA is responsible for the induction of NMDA receptor-mediated postischemic long-term potentiation in the neostriatum (Centonze et al., 2002). It is conceivable that microglia are activated by components released from injured neurons and/or infiltrated from blood. tPA produced and secreted from activated microglia and possibly other cell types may contribute to NMDA receptor-mediated hyperexcitability in the acute phase of neuronal damage (Fig. 6).

Activation of Microglia

Tsirka et al. (1995) have first shown that mice deficient of tPA are resistant to neuronal death induced by excitotoxins. They have also found that microglial activation was significantly attenuated in excitotoxin-injected tPA-deficient mice. Furthermore, an infusion of catalytically inactive tPA into tPA-deficient mice restored microglial activation but not neuronal death after kainate injection (Rogove et al., 1999). By utilization of a domain-deletion approach, Siao and Tsirka (2002) have recently shown that the finger domain of tPA plays a key role in activating microglia during excitotoxic injury. They have concluded that tPA secreted from both

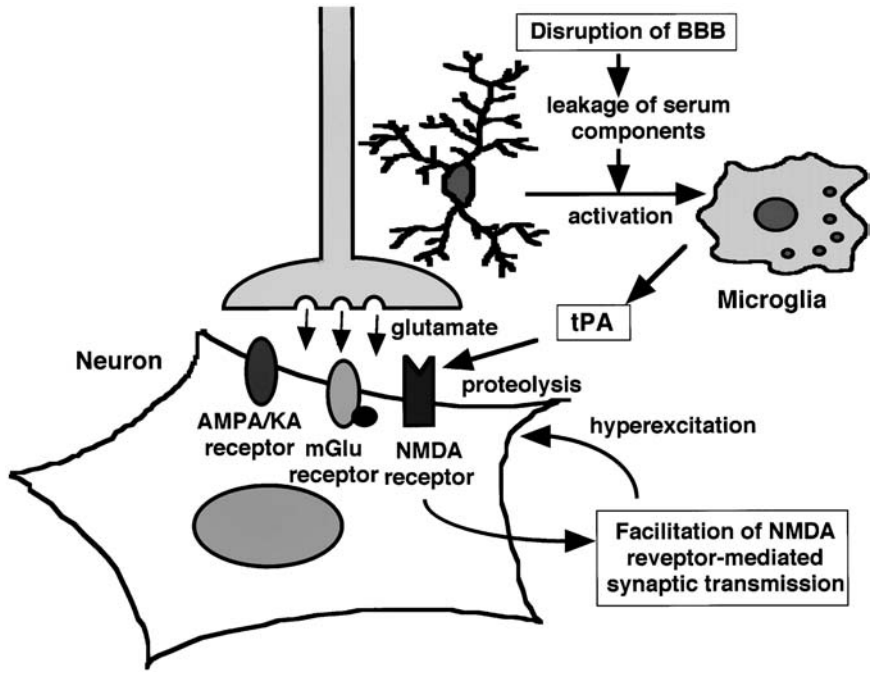

Fig. 6. Secretion of tPA from activated microglia and potentiation of NMDA receptor-mediated synaptic transmission. The blood–brain barrier disruption and subsequent leakage of serum components may contribute to activation of microglia. tPA secreted from activated microglia induce potentiation of NMDA-receptor-mediated synaptic transmission, possibly leading to neuronal death.

neurons and microglia induces an activation of microglia nonproteolytically through its finger domain, which most likely interacts with annexin II, a cell-surface receptor, to initiate an intracellular signaling cascade.

Thrombin is known to activate various cell types, including neurons, astrocytes, and microglia. The thrombin-induced activation of neurons and astrocytes is mediated by proteolytically cleaving PAR-1. Proteolytic activation of PAR-1 results in sequential activation of tyrosine kinase and RhoA, a member of the Ras family of small GTP-binding proteins. Activated RhoA is translocated to the plasma membrane where it can transduce the signal to its effector proteins leading to cellular activation and apoptosis. On the other hand, thrombin induces NO release and iNOS expression in microglia. These effects may be not mediated by proteolytic cleavage of PAR-1 because a peptide agonist could not mimic the effect of thrombin. Thrombin-induced NO release and iNOS expression were significantly suppressed by inhibitors of protein kinase C (PKC), mitogen-activated protein kinase (MAPK), or nuclear factor κB (NF-κB), indicating that thrombin activates microglia via PKC, MAPK, and NF-κB (Ryu et al., 2002). More recently, however, Suo et al. (2002) have demonstrated an essential role in the thrombin-induced microglial activation by utilizing primary cultured microglia from PAR-1 knockout mice.

Migration of Microglia

uPA secreted from microglia has been implicated in extracellular proteolysis, which is required for migration (Nakajima et al., 1992). When uPA binds to its surface receptor (uPAR), uPA activates plasminogen to plasmin, which,

in turn, degrades ECM proteins such as laminin and fibronectin. Plasmin is also capable of activating proenzyme of matrix metalloproteases, which are also known to degrade components of ECM.

Calpains

Calpains, calcium-dependent cysteine proteases, are expressed as proenzymes that undergo autocatalytic processing to yield the mature forms via calcium-dependent mechanism (in micromolar and millimolar concentrations for μ-calpian and m-calpain, respectively). Growing experimental evidence supports the participation of proteolytic cascade mediated by calpains, especially μ-calpain, in pathological functions of microglia.

In multiple sclerosis (MS), an autoimmune demyelination disease, the degradation of myelin proteins results in destabilization of myelin sheath. Calpains have been implicated in myelinolysis because myelin proteins including myelin basic proteins are their substrates and calpains are significantly increased in the white matter from MS patients (Shields et al., 1999). Furthermore, calpains are increasingly expressed in activated microglia, macrophages and mononuclear phagocytes in the white matter of MS patients (Shields et al., 1998). On the basis of these observations, Shields et al. have hypothesized that extracellular calpains secreted from glial cells and inflammatory cells may degrade myelin proteins to produce immunologic fragments engulfed by antigen-processing cells for MHC class II antigen presentation.

Proteasomes

The MHC class I antigen presentation pathway requires the 20S proteasome and two types of activator proteins, PA28 and PA700, to generate the antigenic peptides. The antigenic peptides are transported by a transporter associated with antigen processing into the endoplasmic reticulum, where they become associated with MHC class I molecules and are then translocated to the cell surface to activate cytotoxic T lymphocytes. In response to IFN-γ, three constitutive β subunits of the 20S proteasome are replaced by the immunoproteasome subunits. IFN-γ-induced subunit replacement is implicated in increasing the epitope capacity of 20S proteasome.

It has been suggested that microglia are involved in MHC class I-mediated antigen presentation during viral infection (Kolson et al., 1998). Recently, Stohwasser et al. (2000) characterized the dynamics of the 20S/26S proteasome subunit composition of primary cultured murine microglia and BV-2 cells in response to IFN-γ and LPS. Both IFN-γ and LPS induced immunoproteasomes in primary cells, whereas BV-2 cells responded only to IFN-γ. These observations support the idea that microglia play a pivotal role in MHC class I-mediated antigen presentation during viral and bacterial infections in the CNS.

Caspases

Caspases have received a great deal of attention because of their essential roles for the execution step in apoptosis. Caspases are synthesized as proenzymes and activated by proteolytic cleavage at Asp-X sites. The mature caspase has a cysteine residue at its active site and proteolytically cleave substrates at their Asp-X sites. Although at least 10 family members are known in mammals, caspase-3 activation plays a central role in apoptosis to cleave various important cytoplasmic and nuclear proteins.

We have reported that relatively low concentrations of neurotoxins such as 6-hydroxydopamine and methylmercury induce apoptosis of primary cultured rat microglia mainly through activation of caspase-3-like proteases and partially through endosomal/lysosomal proteases (Takai et al., 1998; Nishioku et al., 2000). The apoptosis of microglia induced by 6-hydroxydopamine and methylmercury compromises the host defense system

and homeostasis of the CNS and may have an important pathogenic implication of Parkinson's disease and Minamata disease, respectively. Recently, it has been also reported that overactivation causes microglia to undergo apoptosis through an activation of caspases (Kingham and Pocock, 2000; Lee et al.,2001; Liu et al., 2001). These observations strongly suggests that overactivation-induced apoptosis of microglia is an auto-regulatory mechanism which downregulates the number of activated microglia after termination of the pathological stimuli in the CNS.

Matrix Metalloproteases

Matrix metalloproteases (MMPs), which are essential to maintenance and restructuring of the ECMs, consist of approx 20 homologues including gelatinases, collagenases, and stromelysins. MMPs usually present in latent forms are activated by autocatalysis or cleavage by membrane-type MMPs or plasmin. Reactive oxygen radicals also activate MMPs.

In the CNS, MMP-2 (gelatinase A) and MMP-9 (gelatinase B) are secreted by microglia and astrocytes as active forms (Colton et al., 1993; Chauvet et al., 2001). MMP-2 and MMP-9 secreted by microglia contribute to inflammation and tissue degradation through damage to the blood–brain barrier and processing proinflammatory cyokines such as interleukin 1-β. Recently, Selkoe's group has screened certain neuronal and nonneuronal cell lines to ascertain whether they constitutively release proteases capable of degrading Aβ (Qiu et al., 1997). Among the cells examined, they found that BV-2 produces the most potent Aβ-degrading activity, which was completely blocked by MMP inhibitor. After purification and characterization of Aβ-degrading protease secreted from microglia, they concluded that it is insulin-degrading enzyme, a thiol metalloendopeptidase that degrades small peptides such as insulin, glucagons, and atrial natriuretic peptide (Qiu et al., 1998). Insulin-degrading enzyme was also found to be capable of mediating the oligomerization of Aβ. On the basis of these observations, microglia play an important role in the clearance of Aβ from the extracellular space in the CNS.

Summary

The activities of microglial proteases are strictly regulated by the level of their transcripts or their endogenous inhibitors in the CNS. Most of microglial proteases are upregulated after cellular activation during neuropathological changes. Microglial proteases are involved in antigen presentation, neuronal death, potentiation of glutamatergic neurotransmission, migration, inflammation, clearance of Aβ, activation of microglia, and apoptosis of microglia. The growing understanding of the proteolytic systems that are directly or indirectly associated with microglial functions could also contribute to the development of protease inhibitors as novel neuroprotective agents.

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