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Neurotrophic Factors Regulate Cathepsin S in Macrophages and Microglia: A Role in the Degradation of Myelin Basic Protein and Amyloid β Peptide

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Abstract

Background: Cathepsin S is a member of the family of cysteine lysosomal proteases preferentially expressed in macrophages and microglia and is active after prolonged incubation in neutral pH. Upon activation of macrophages by a number of inflammatory mediators, there is an increase in secreted cathepsin S activity accompanied by a decrease in cellular cathepsin S activity and protein content, as well as a decrease in cathepsin S mRNA. The decrease in cathepsin S mRNA and protein at the cellular level is in contrast to the response observed in some in vivo scenarios.
Materials and Methods: We investigated the effect of basic fibroblast growth factor (bFGF) and nerve growth factor (NGF), two growth factors present during cell injury and inflammation but not known to activate macrophages and microglia, on the expression of cathepsin S, cathepsin B, and cathepsin L mRNAs in these cells, and on cathepsin S activity. We then tested the ability of cathepsin S to degrade myelin basic protein, and amyloid β peptide at both acidic and neutral pH.
Results: Basic FGF and NGF treatment of macrophages and microglia significantly increased the levels of cathepsin S, B, and L mRNAs (2- to 5-fold). Basic FGF also increased cathepsin S activity intra- and extracellularly. Recombinant human cathepsin S was able to degrade myelin basic protein and monomeric and dimeric amyloid β peptide at both acidic and neutral pH, as well as to process human amyloid precursor protein generating amyloidogenic fragments.
Conclusions: These data suggest that bFGF and NGF may be the molecular signals that positively regulate the expression and activity of cysteine lysosomal proteases (cathepsin S in particular) in macrophages and microglia in vivo, and that there is an interplay between these factors and the activators of inflammation. Disruption of the balance between these two categories of signals may underlie the pathological changes that involve cysteine proteases.

Introduction

The role of "activated" macrophages and microglia during inflammatory reactions in the central and peripheral nervous system has been well established (1,2). Typical activation signals include bacterial endotoxins or inflammatory cytokines, e.g., interferons, interleukins, colony-stimulating factors, and tumor necrosis factor. Following activation, macrophages can secrete a wide variety of biologically active products, including proteases, that are important mediators of the tissue destruction and fibrosis characteristic of chronic inflammation (3,4). We have previously shown that the cysteine lysosomal protease, cathepsin S, is secreted from macrophages

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and microglia by the above activation signals (5,6). Cathepsin S retains activity after prolonged incubation at neutral pH, whereas all the other cysteine lysosomal proteases, e.g., cathepsins B, H, L, and O, become irreversibly inactivated (7,8). Thus, cathepsin S is a good candidate for tissue destruction and remodeling of the extracellular matrix (ECM) in both acidic and non-acidic extracellular environments (9).

We and others have demonstrated that cathepsin S expression is restricted to cells of the mononuclear-phagocytic system (5,10). In the central nervous system (CNS), microglia are responsive in many pathological scenarios of neuronal degeneration whereby an inflammatory process may or may not be evident, e.g., in Alzheimer's disease, multiple sclerosis, AIDS, Parkinson's disease, amyotrophic lateral sclerosis, and stroke (2,11,12). In the peripheral nervous system (PNS) and the CNS, macrophages and microglia play a key role during Wallerian degeneration (11). Cathepsin S is able to degrade a number of components of ECM including those found in the CNS, such as neurocan, phosphacan, and basement membrane heparan sulfate proteoglycan (5,6,13–15). Thus, secreted cathepsin S from mononuclear–phagocytic cells may play a role during a multitude of inflammatory and degenerative processes in the periphery and in the brain (2,11,12).

We have shown that secretion of cathepsin S from macrophages and microglia is positively regulated by activating inflammatory stimuli; however, these agents cause a decrease in its mRNA, protein, and cellular protease activity (6). Also, following entorhinal cortex lesion of rat brain, a classical in vivo paradigm for anterograde neuronal degeneration and microglial activation, there is increased mRNA for cathepsins S, B, and L in the outer molecular layer of the ipsilateral hippocampus, with an overwhelming response by cathepsin S, that is localized to microglia (5). In addition, others have shown that cathepsin L can be up-regulated in murine thioglycolate-elicited peritoneal macrophages; in this in vivo scenario the agents directly responsible are also unknown (16). Therefore, it is intuitive that factors other than those mentioned above, must exist to up-regulate cathepsin levels inside cells, and that they may be associated with macrophage activation. Here we examine the potential regulation of cathepsin S expression and activity in macrophages and in microglia by two prototypic neurotrophic factors that are known to have different signal transduction properties than pro-inflammatory agents. We demonstrate that cathepsin S, cathepsin B, and cathepsin L mRNA expression is positively regulated by both bFGF and nerve growth factor (NGF). We also show that cathepsin S has the ability to degrade myelin basic protein (MBP), and amyloid β peptide in vitro, and propose a role for cathepsin S in neurodegenerative processes.

Materials and Methods

Cell Culture and Treatments

Murine macrophage-like cell line RAW 264.7 (gift from Dr. S. Gandy, Nathan Kline Institute at NYU, Orangeburg, NY) and murine microglial cell line N13 (gift from Dr. P. Ricciardi-Castagnoli, University of Milan) were cultured for enzyme and mRNA experiments at 1.5×10^5 cells/well in 24-well plates or at 2.0×10^6 cells in 60-mm dishes, respectively, for 24 hr as described previously (6). Treatments were done in macrophage serum-free medium (MSFM) (Gibco, BRL, Gaithersburg, MD) in the absence or presence of bFGF (gift from Dr. D. Moscatelli, NYU Medical Center, New York) or NGF (gift from Dr. J. Musacchio, NYU Medical Center).

RNA Isolation and Northern Blot Analysis

Total RNA was isolated using the acid guanidinium thiocyanate (GTC)-phenol-chloroform extraction method(17) and was fractionated in agarose gels and transferred onto Gene Screen Plus membranes (DuPont NEN, Boston, MA) as described previously (6). Membranes were hybridized to [^{32}P]-radiolabeled cDNA probes as described previously (6,18). The membranes were exposed to a phosphor screen and quantitative analysis of mRNA bands was performed on a phosphorimager (Molecular Dynamics, Sunnyvale, CA); results were standardized to probed 18S rRNAs.

Cathepsin S Activity Determination

Enzymatic assays of conditioned media (C.M.) and cellular lysates were performed as previously described (5,6). Cell lysates were subjected to a 1-hr neutral pH incubation step prior to cathepsin S activity determination as previously described(6); this step has been shown to inactivate the other lysosomal proteases with substrate specificity similar to that of cathepsin S (8,15). All samples were incubated with the substrate, *N*-carbobenzoxy-

Phe-Arg-7-amido-4-methylcoumarin (Z-FR-AMC, Sigma, St. Louis, MO) in the presence of 2 mM EDTA, 2 mM DTT, 0.01% Triton X-100, 1 μM PMSF, and 5 μM pepstatin A, and the product formed was detected with a Fluoroskan II plate reader as previously described (6). Inclusion of 10 μM of the highly selective active site-directed cysteine protease inhibitor E64 (Sigma) in parallel samples was used to specifically determine cathepsin S activity.

Proliferation Assay

RAW 264.7 macrophage and N13 microglial cell lines were initially cultured at 1.5–2.0 $\times$ 10^5 cells/per 35-mm dish in Dulbecco's modified Eagle medium (DMEM) + 10% fetal bovine serum (FBS) for 18 hr. Cells were washed two times with phosphate-buffered saline (PBS) and serum starved for 6–8 hr in MSFM (Gibco, BRL). Medium was replaced with fresh medium containing no addition, 1 ng/ml bFGF, 25 ng/ml NGF, or 10% FBS, and cells were cultured for the next 2 days. At 1 and 2 days after treatments, the cells were harvested with trypsin and plates were washed vigorously twice with PBS and checked under a microscope to ensure removal of all cells. Trypsin and PBS washes were combined, cells were centrifuged and resuspended in 500 ml of PBS, and 10-ml aliquots were counted with a hemacytometer. Data represent total cell number retrieved from the dishes.

Proteolysis of Myelin Basic Protein by Cathepsin S

Ten micrograms of bovine MBP (Sigma) was incubated with recombinant human cathepsin S (gift of Dr. D. Bromme, Mount Sinai School of Medicine, New York), at a 1:30 and 1:300 w/w enzyme-to-substrate ratio, in an acidic buffer (100 mM sodium phosphate, pH 6.5) or a neutral buffer (100 mM TrisCl, pH 7.5) containing 2 mM EDTA, 2 mM DTT, and 0.01% Triton X-100. The digestions were performed for 15 or 150 min at 37°C. Mock digestions and digestions in the presence of E64 were performed as negative controls. The reaction products were resolved on 4–20% gradient SDS polyacrylamide gels and visualized by silver staining.

Amyloid β Peptide Proteolysis by Cathepsin S

Lyophilized, high-performance liquid chromatography (HPLC)-purified, $A\beta^{(1-40)}$ peptide was resuspended in 50% acetonitrile/0.01% trifluoroacetic acid (TFA) at 1 mg/ml, and 10-μg aliquots were lyophilized and diluted in acidic or neutral pH buffers (described above) to a final concentration of 20 μM, and digested with recombinant, human cathepsin S (1:100 enzyme to substrate molar ratio) at 37°C for 1 hr. The reaction products were resolved on 15% SDS-polyacrylamide gels and visualized by silver staining or Western analysis with the SP28 rabbit polyclonal antibody.

Results

Treatment of macrophage and microglial cells with agents that are known to produce their activation results in increased secretion of cathepsin S, but negative regulation of mRNA expression for cathepsins S, B, and L (6). We examined whether cathepsins can be regulated differently by neurotrophic factors that are possibly mitogenic for microglia and macrophages and are known not to produce their activation. To date, it is not known whether neurotrophic growth factors can regulate mRNA expression of cysteine lysosomal proteases.

We examined the effect of bFGF and NGF on the mRNA transcripts of cathepsins S, B, and L in N13 microglial and RAW 264.7 macrophage cell lines. Both bFGF and NGF dose-dependently increased the levels of all three mRNAs. This effect is opposite that by LPS, IFN-γ, TNF-α, IL-1α, IFN-α, and GM-CSF, which are known to promote activation of microglia and macrophages (6). Treatment of RAW macrophages for 24 hr with bFGF increased the levels of cathepsin S, B, and L mRNAs about 1.5-fold ($p \leq 0.05$; Fig. 1A, B). Basic FGF had a more potent and dose-dependent stimulatory effect on cathepsin mRNA expression in microglial cells compared to macrophages (Fig. 1C, D). Treatment of N13 microglial cells with bFGF increased cathepsin S, B, and L mRNA levels about 3.2- to 4.0-fold ($p \leq 0.05$). Furthermore, the treatment of microglia with bFGF produced a biphasic effect with maximal increases in cathepsin mRNAs at 1 ng/ml bFGF.

We examined the effects of NGF on cathepsin mRNA levels in macrophages and microglia. NGF treatment led to an increase in all three transcripts in both macrophages and microglia and was more effective than bFGF (Fig. 2). Again, the effect was biphasic with an intermediate dose, being more stimulatory than higher doses of NGF (Fig. 2B, D). The biphasic response

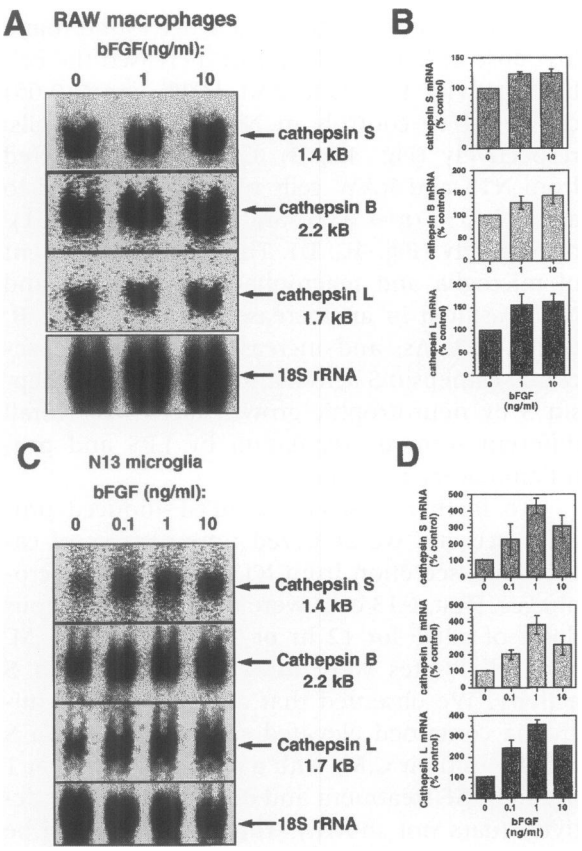

Fig. 1. Increased cathepsin S, B, and L mRNA after bFGF treatment of macrophages and microglia for 24 hr. RAW macrophages (A, B) and N13 microglial (C, D) cells were treated for 24 hr in the presence of various doses of bFGF, total RNA was isolated, and specific mRNAs for cathepsins S, B, and L were detected by Northern analysis as described in Materials and Methods. Data shown in A and C are representative results; data in B and D are mean ± SEM from three experiments performed independently. Graphs represent densitometry of specific bands performed on phosphorimager.

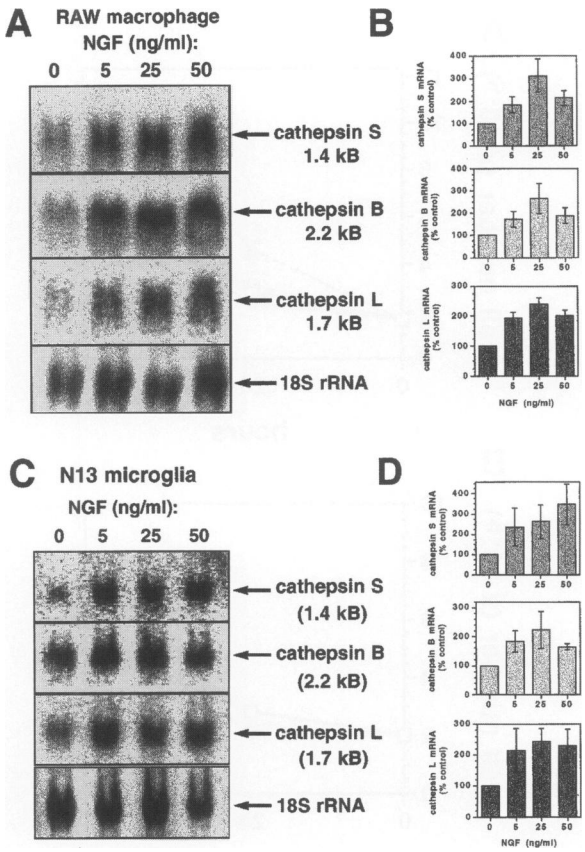

Fig. 2. Increased cathepsin S, B, and L mRNA after NGF treatment of macrophages and microglia for 24 hr. RAW macrophages (A, B) and N13 microglial (C, D) cells were treated for 24 hr in the presence of various doses of NGF, total RNA was isolated, and specific mRNA for cathepsins S, B, and L were detected by Northern analysis as described in Materials and Methods. Data shown in A and C are from a representative experiment; data in B and D are mean ± SEM from three independent experiments. Graphs represent densitometry of specific bands performed on phosphorimager.

of cathepsin B, L, and S mRNAs to bFGF and NGF suggests the possibility of dual transcriptional regulation of these enzymes in vivo.

Since the increased mRNA for cathepsins produced by these neurotrophic growth factors could be due to an increase in cell proliferation, the effects of bFGF and NGF on macrophage and microglial cell number were examined. After serum starvation for 9–12 hr RAW macrophages and N13 microglial cells were incubated in MSFM in the presence or absence of bFGF or NGF. Cells treated with 10% FBS were considered positive controls. Under conditions where treatment with bFGF resulted in elevated cathepsin mRNA levels, there was no significant increase in cell numbers after 24 or 48 hr compared to controls treated with MSFM alone (Fig. 3A, B). In agreement with this finding is the observation that bFGF is not a direct mitogen for isolated microglia (19). In addition, treatment of N13 cells with 1 ng/ml bFGF and 100 ng/ml heparin had no effect on cell proliferation (data not shown). Treatment of N13 microglial cells with NGF under the same conditions that elevated cathepsin mRNA levels demonstrated no effect on cell number after 24 hr, but displayed a small (23%) increase in microglial cell number after 48 hr of treatment compared to treatment with MSFM alone (Fig. 3B). RAW macrophages treated with NGF demonstrated no change in cell

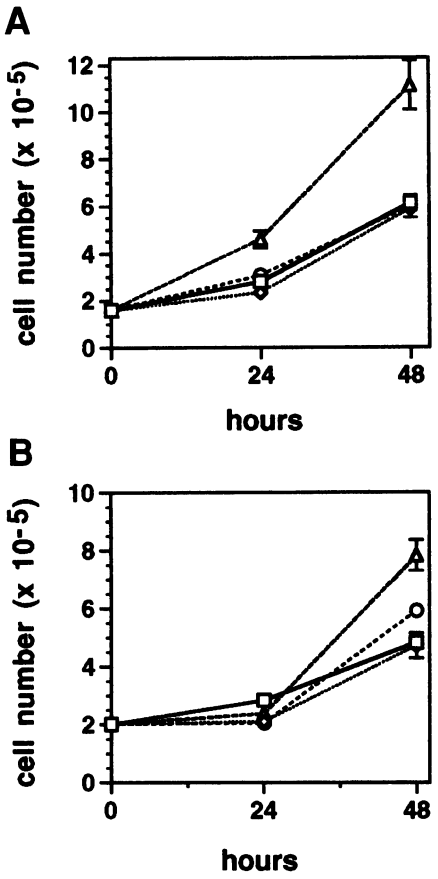

Fig. 3. Effect of bFGF and NGF on macrophage and microglial cell line proliferation. RAW macrophages (A) and N13 microglia (B) initially plated in DMEM and serum for 18 hr at subconfluent cell densities were then serum starved for 6–8 hr followed by treatment with fresh MSFM alone (open squares), 10 ng/ml bFGF (open circles), or 1 ng/ml of bFGF in MSFM (open diamonds), 25 ng/ml of NGF in MSFM (open circles), or Dulbecco (Db) medium with 10% FBS (open triangles). After 1 or 2 days, the cells were harvested and counted as described in Materials and Methods. Data represent average of duplicate plates with calculated standard errors.

number after 24 or 48 hr of treatment (Fig. 3A). Therefore, the dramatic increase in cathepsin mRNA levels after 24-hr treatment of N13 microglia and RAW macrophages with bFGF and NGF could not be accounted for by enhanced proliferation of these cells.

We next analyzed whether bFGF could stimulate the secretion of cathepsin S activity from microglia and macrophages. After treatment of cells for 24 hr with 0.1, 1, and 10 ng/ml of bFGF, both the cellular and the secreted cathepsin S activity by microglia and macrophages were elevated. In correlation with the observed increase in mRNA levels for cathepsin S, we found that a low dose of bFGF (0.1 ng/ml) increased the cellular activity to 170% and 160% ($p \leq 0.05$) compared to controls in N13 and RAW cells, respectively (Fig. 4A, B). Cathepsin S secreted from N13 and RAW cells was also increased to about 145% ($p \leq 0.05$) and 470% ($p \leq 0.001$), respectively (Fig. 4C, D). Thus, 24-hr treatment of microglia and macrophages with bFGF and NGF resulted in an increase in cathepsin S, B, and L mRNAs, and increased cellular and secreted cathepsin S activity. Regulation of cathepsin S by neurotrophic growth factors is overall different from its regulation by LPS and proinflammatory cytokines.

To further characterize bFGF-induced protease activity, we analyzed time courses of cathepsin S secretion from N13 and RAW macrophages. First, N13 cells were treated with various doses of bFGF for 12 hr or 24 hr and the C.M. and cell lysates were assayed for cathepsin S activity. We observed that after 12 hr N13 microglia contained elevated secreted cathepsin S activity in their C.M. with a peak of 1.6-fold at 1 ng/ml bFGF treatment and decreased cellular activity (data not shown). This decrease could be due to the increased secretion of cathepsin S from intracellular stores. However, after 24 hr of treatment, bFGF caused a significant increase in cellular cathepsin S activity to 171% and 152% compared to controls. The level of cathepsin S activity in C.M. from N13 cells treated for 24 hr was 2- to 3-fold higher than after 12 hr of treatment, suggesting continual secretion (data not shown). The increase in cellular cathepsin S activity induced by bFGF after 24 hr correlates well with the increased mRNA levels in these cells (Fig. 1B).

We also followed the time course of cathepsin S activity in RAW macrophages after bFGF treatment. As in N13 cells above, cellular and secreted cathepsin S activity increased after 24 hr of treatment (Fig. 5). There is a further elevation of enzyme activity after 48 hr of treatment compared to that found at 24 hr (Fig. 5A). This may be due to increased synthesis of cathepsin S protein since its mRNA is increased after 24 hr (Fig. 1A). After 48 hr of bFGF treatment, cathepsin S activity is relatively decreased inside cells compared to control cells (Fig. 5A), which is consistent with the relative decrease in cathepsin S mRNA after 48 hr (data not shown). Therefore, cellular cathepsin S activity in macrophages correlates well with the mRNA levels. This also suggests that the increased expression by bFGF is not

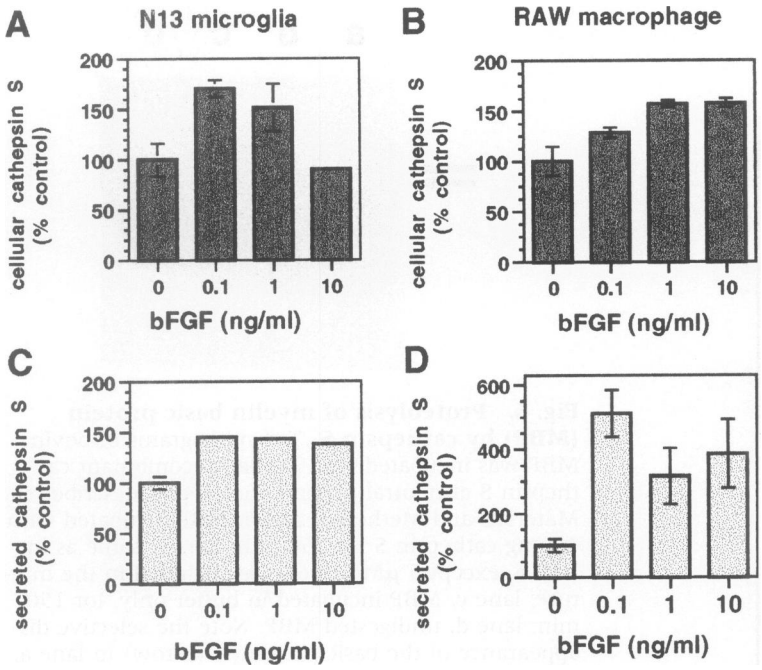

Fig. 4. Increased cathepsin S protease activity in cells and conditioned medium of bFGF-treated macrophages and microglia. Cells were treated for 24 hr with various doses of bFGF and cathepsin S protease activity was measured in cells (A, B), and C.M. (C, D) as described in Materials and Methods. Data represent mean ± SEM of triplicate determinations measuring the E64-inhibitable cathepsin S activity. Activity in the absence of bFGF treatment is taken as control (100%).

an irreversible process. It is of interest that the level of secreted cathepsin S activity in macrophage C.M. was increased 5- to 6-fold after 48 hr (Fig. 5B), although the cellular activity and mRNA returned to basal levels by that time. These results suggest that the high level in the C.M. represents accumulation of secreted cathepsin S activity that remains stable at neutral pH.

The list of potential substrates for cathepsin S is growing and includes many ECM proteins suitable for a secreted stable protease (5,13–15). Particularly in the CNS, potential substrates for cathepsin S include two brain chondroitin sulfate proteoglycans, Neurocan and Phosphacan, and basement membrane heparan sulfate proteoglycans (5,6). We tested the MBP from bovine brain as a substrate for cathepsin S, since microglia and macrophages play a pivotal role in establishing the pathology of various demyelinating diseases (20). Commercially supplied MBP was only 50% pure, and contained a major band of 18.5 kDa, which represents the basic protein. The basic protein selectively dissapeared during the course of a 150-min incubation with recombinant cathepsin S at pH 7.5 (Fig. 6). A similar selective decrease of the basic protein band was also seen upon digestion at pH 6.5 (data not shown). This finding suggests a possible role for cathepsin S in the breakdown of myelin during various demyelinating neuropathies.

Activated microglia surround both diffuse and mature senile plaques in the brains of patients with Alzheimer's disease (21–23). Microglia are known to secrete proteases capable of Aβ peptide clearance (24). We examined the ability of cathepsin S to degrade Aβ peptide. Soluble A$\beta^{(1-40)}$ peptide was incubated with cathepsin S at 1:100 enzyme-to-substrate molar ratio at both acidic and neutral pH. The reaction mixtures were analyzed by Western blotting with the SP28 antiserum (Fig. 7), or by silver staining (data not shown). Cathepsin S was able to degrade most of the monomeric Aβ peptide and substantial amounts of the dimeric peptide within 1 hr at acidic pH (Fig. 7, lane 2). At neutral pH both the monomeric and dimeric peptides were substantially degraded to the same extent (Fig. 7, lane 3). These data suggest that cathepsin S may take part in the clearence of Aβ peptides in vivo, either intracellularly or extracellularly.

Discussion

In this study we have determined that the neurotrophic factors bFGF and NGF can positively regulate the expression of cysteine lysosomal proteases cathepsins B, L, and S in macrophages and microglia, as well as increase secretion of cathepsin S. Murine fibroblasts stimulated with phorbol ester, platelet-derived growth factor

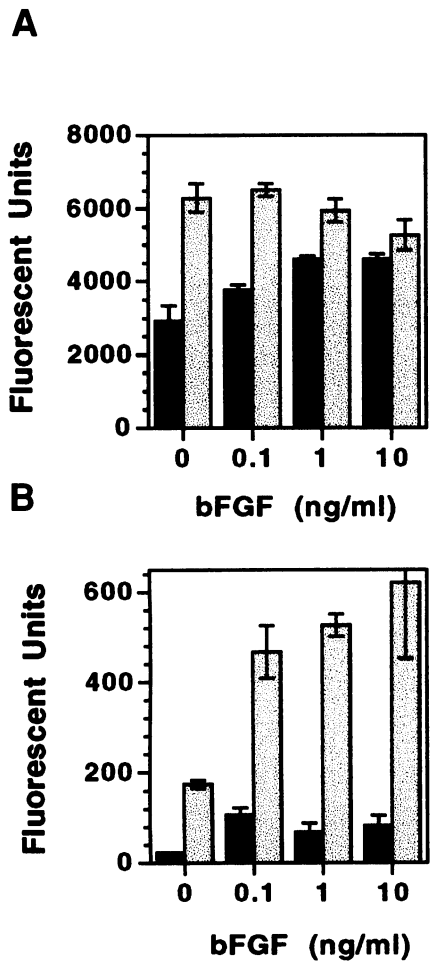

Fig. 5. Time-course analysis of bFGF-induced cathepsin S protease activity in macrophages. RAW macrophage cells were treated with various doses of bFGF for 24 (solid bars) or 48 hr (shaded bars) and cathepsin S protease activity was measured in cells (A), and C.M. (B), utilizing the fluorogenic substrate Z-FR-AMC as described in Materials and Methods. Data represent mean ± SEM of measured E64-inhibitable cathepsin S activity of triplicate samples each with triplicate determinations. Cathepsin S activity is expressed in fluorescent units and activity detected in the absence of bFGF treatment is taken as control.

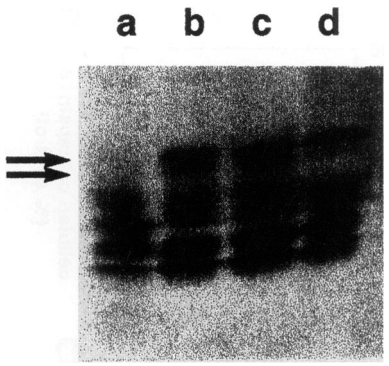

Fig. 6. Proteolysis of myelin basic protein (MBP) by cathepsin S. Ten micrograms of bovine MBP was incubated with human recombinant cathepsin S at neutral pH and analyzed as described in Materials and Methods. Lane a, MBP incubated with 300 ng cathepsin S for 150 min; lane b, same as in lane a, except 5 μM of E64 was included in the mixture; lane c, MBP incubated in buffer only, for 150 min; lane d, undigested MBP. Note the selective disappearance of the basic protein(s) (arrow) in lane a, compared to controls.

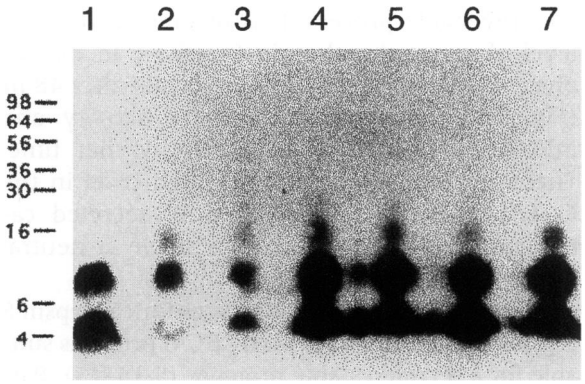

Fig. 7. Proteolysis of Aβ peptide by cathepsin S in vitro. HPLC-purified aliquots (10 μg each) of A$\beta^{(1-40)}$ peptide were incubated with recombinant, human cathepsin S (1 hr, 37°C) at 1:100 enzyme to substrate molar ratio, and analyzed by Western blotting using the SP28 antiserum. Lane 1, undigested Aβ; lane 2, Aβ and cathepsin S at pH 6.5; lane 3, Aβ and cathepsin S at pH 7.5; lane 4, Aβ, cathepsin S, and E64 at pH 6.5; lane 5, Aβ, cathepsin S, and E64 at pH 7.5; lane 6, Aβ mock digestion at pH 6.5; lane 7, Aβ mock digestion at pH 7.5.

(PDGF), NGF, and FGF can increase their secretion of an inactive cathepsin L precursor (major excreted protein), suggesting possible regulation of cathepsins by growth factors (25–27). Cathepsin S appears to have restricted expression within cells of the mononuclear–phagocytic system. These highly secretory cells are essential for orchestration of the complex events occurring during neuronal degeneration and regeneration, as well as chronic inflammatory processes, e.g., arthritis, atherosclerosis, wound healing, and emphysema (11). Previously, we have demonstrated that following entorhinal cortex lesion of rat brain, a classical paradigm for anterograde neuronal degeneration with a well-characterized microglial response, there was an increase of mRNA transcripts for cathepsins L and S in the outer molecular layer of the ipsilateral dentate

gyrus with an overwhelming response for cathepsin S mRNA (5). Our data here support those findings and suggest that NGF and bFGF may be the molecular signals regulating the expression of cathepsin S and cathepsin L mRNAs, especially since following entorhinal cortex lesion, bFGF immunoreactivity is increased in the outer molecular layer of the ipsilateral dentate gyrus (28).

Regulation by growth factors has important implications in the development and pathology of the CNS. Basic FGF is a well-known neurotrophic factor that has been immunocytochemically localized to neurons, and acts directly on a broad range of neuronal populations in the developing and adult CNS to promote their proliferation, differentiation, functional maintenance, and survival (29–31). In addition to neurons, bFGF has also been demonstrated in astrocytes and microglia and is released by all three of these cultured cell types in vitro (32). Following entorhinal cortex lesions, increased bFGF immunoreactivity was found in the astrocytes and surrounding ECM (28), which would support a paracrine mechanism for increased cathepsin mRNA expression in microglia. Macrophages also produce bFGF, which in the brain can potently stimulate the proliferation of perineuronal satellite cells, endothelial cells, vascular pericytes, macrophages, and glial cells (33). Microglia contain specific receptors for bFGF on their cell surface (34,35), and in a model of autoimmune inflammation of the CNS it was found that bFGF and FGFR-1 are induced in activated microglia and brain macrophages (36). Furthermore, the phagocytic properties of microglia are not affected by bFGF (20).

In the CNS, NGF is synthesized and released from both astrocytes and LPS-stimulated microglia (37–39). Extracellular NGF can be chemotactic for, as well as taken up by, microglia (37,38,40). Thus, microglia must have specific receptors for NGF, which may promote gene expression, proliferation, or both. Following a peripheral nerve lesion, the recruitment of macrophages is important for Wallerian degeneration and for NGF synthesis by non-neuronal cells, which is essential for sensory axon maintenance and regrowth (41). Macrophages themselves can synthesize NGF upon stimulation with LPS (38,42), but also produce IL-1 upon their activation, which increases the expression of NGF in the Schwann and fibroblast-like cells associated with peripheral nerves (43). During CNS or peripheral nerve injury, inflammation in the brain, or neurodegenerative diseases, bFGF and NGF are released and have the potential to act upon microglia and macrophages that are migrating and actively responding in these scenarios.

Our data are the first evidence that bFGF regulates mRNA expression of cysteine lysosomal proteases. Along this line, we hypothesize that growth factors like bFGF and NGF released during injury from neurons and astrocytes in vivo interact with microglia and macrophages, and lead to an increase of cathepsin S, B, and L mRNA expression. Basic FGF also increases cathepsin S protease activity in these cells and increases the secretion of active enzyme. Activation of these cells by inflammatory mediators in turn decreases cathepsin S, B, and L mRNAs, while increasing secretion of cysteine lysosomal protease activity (6). Therefore, there must be a fine balance between regulation by growth factors and by pro-inflammatory agents during neuronal degeneration. The order in which these two types of signaling molecules act on macrophages and microglia is currently unknown, but alteration in their equilibrium may contribute to pathological states.

One of the major functions of microglia and macrophages is phagocytic removal of dead cells or cell remnants during brain development and after injury in adult brain (44). The ability of cathepsin S to degrade MBP at acidic and neutral pH and its potential to be secreted together raise the possibility of involvement of cathepsin S in demyelinating diseases. Microglia and macrophages play a pivotal role in establishing these neuropathies, functioning both as antigen-presenting cells as well as effector cells that actively strip off myelin sheets (2,21,45). Although the mechanism by which the myelin sheath is degraded in these diseases is not known, increased activity of both acid and neutral proteases has been demonstrated in tissues from experimental allergic encephalomyelitis animals and multiple sclerosis patients in correlation with the disappearance of myelin proteins (21). During Wallerian degeneration, a model for secondary demyelination in the PNS, rapid loss of myelin-specific proteins is accompanied with an increase in many lysosomal enzymes (11,46). In addition, NGF levels have been found to be elevated in the serum of patients and correlated to the degree of inflammation associated with autoimmune disorders, such as multiple sclerosis, rheumatoid arthritis, and systemic lupus erythematosus (42). Our findings that cathepsin S degrades MBP in vitro, an important component of the myelin

sheath, and that cathepsin S mRNA expression and secretion are regulated by bFGF and NGF and by inflammatory agents, suggest a role for cathepsin S during inflammatory processes associated with neuronal degeneration (6). Further in vivo studies are required to confirm this hypothesis.

Activated microglia surrounding senile amyloid plaques and a low-level inflammatory response are characteristic for Alzheimer's disease (22,23). Immunoreactive cathepsin S is increased in Alzheimer's and Down syndrome brains, and is localized in the vicinity of activated microglia and degenerating neurons (22). We demonstrate that cathepsin S can degrade monomers and dimers of the Aβ peptide in vitro. This suggests that cathepsin S may assist in the clearance of intracellularly formed Aβ, or soluble Aβ extracellularly, and modulate the levels of the peptide at the very initial stages of peptide aggregation, which in turn might have an effect on Aβ neurotoxicity (23).

In summary, we have shown that growth factors regulate the expression of cysteine lysosomal proteases in macrophages and microglia. More specifically, in response to bFGF and NGF, cathepsin S mRNA and enzyme activity are increased. This fact, and the ability of cathepsin S to degrade MBP and Aβ both at acidic and neutral pH, suggest that disregulation of these factors may accompany pathological changes during inflammatory and degenerative processes in the periphery and in the brain.

Acknowledgments

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