Induction of caspase-11 by inflammatory stimuli in rat astrocytes: lipopolysaccharide induction through p38 mitogen-activated protein kinase pathway[1]

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Abstract Caspase-11 plays a crucial role in both inflammation and apoptosis. Caspase-11 not only activates caspase-1, that is required for the maturation of proinflammatory cytokines such as interleukin (IL)-1 and IL-18, but also activates caspase-3, leading to cellular apoptosis under pathological conditions. Here, we cloned the rat homolog of caspase-11, and investigated its inducibility by inflammatory stimuli and signal transduction pathways involved. Deduced amino acid sequence of rat caspase-11 showed 88.7% similarity to mouse caspase-11, and in vitro translation of rat caspase-11 cDNA yielded approximately a 43 kDa polypeptide, which was in agreement with predicted protein size generated from full-length rat caspase-11 cDNA. The expression of caspase-11 was strongly induced at both mRNA and protein levels by inflammatory stimuli such as lipopolysaccharide (LPS), interferon-γ, and tumor necrosis factor-α in C6 rat glial cells as well as primary astrocytes. LPS induced activation of both p38 mitogen-activated protein kinase (MAPK) and extracellular signal-regulated kinase (ERK) in C6 cells. However, SB203580 (specific inhibitor of p38 kinase), but not PD98059 (specific inhibitor of ERK kinase), inhibited LPS induction of caspase-11, indicating that induction of caspase-11 by LPS in astrocytes was mediated through the p38 MAPK pathway. Inflammatory induction of caspase-11 in astrocytes may play an important role in both inflammatory responses involving these cells and auto-regulatory apoptosis of activated astrocytes in inflammatory sites.

Key words: Caspase-11; Astrocyte; Inflammation; Lipopolysaccharide; Mitogen-activated protein kinase

1. Introduction

Caspases play a central role in cellular apoptosis [1]. A variety of apoptotic stimuli lead to the activation of initiator caspases, which in turn triggers the caspase cascade and ultimately results in apoptotic cell death. Among over a dozen caspases identified so far, caspase-11 has been first characterized as an activator of caspase-1 in mice [2]. Caspase-11-deficient mice are resistant to endotoxic shock induced by bacterial lipopolysaccharide (LPS) and fail to produce mature interleukin-1 (IL-1) in response to LPS. Also, embryonic fibroblasts from caspase-11-deficient mice are resistant to apoptosis by ectopic expression of caspase-1, indicating that caspase-11 in an upstream activator of caspase-1 [3]. The human homolog of mouse caspase-11 has not been conclusively identified, although human caspase-4 and -5 are considered to be good candidates [4]. It has been claimed in a recent report that caspase-5 is the human homolog of caspase-11 based on LPS inducibility [5]. Nevertheless, the authentic homolog of mouse caspase-11 in any other species has not been documented. Caspase-11 has been proposed to play an important regulatory role in both apoptosis and inflammatory responses [6]. Activation of caspase-11 was crucial for the activation of caspase-1 [3], however, recent works demonstrated that caspase-11 also activated caspase-3 under pathological conditions [7]. Caspase-11 has been shown to carry out an essential function in apoptotic death of oligodendrocytes and neuronal cells [6–8]. Caspase-11-deficient mice were partly resistant to the induction of experimental allergic encephalomyelitis (EAE) [6] and showed a reduced number of apoptotic cells after middle cerebral artery occlusion [7]. The expression of caspase-11 was increased by ischemia and hypoxia [8,9], and this increase in the expression was believed to lead to the activation of caspase-11 [7]. Recombinant procaspase-11 has been shown to auto-process itself in vitro, supporting that an elevated concentration of caspase-11 in stimulated cells may be sufficient for its auto-activation. Moreover, we have previously shown that the expression of caspase-11 is induced by inflammatory stimulation in microglia, and caspase-11 induction constitutes a NO-independent apoptotic pathway [10].

Mitogen-activated protein kinase (MAPK) pathways have been implicated in inflammatory activation of astrocytes [11–14]. MAPKs consist of three subgroups: p38 MAPK, extracellular signal-regulated kinase (ERK), and c-Jun N-terminal

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[1] Nucleotide sequence data reported are available in the GenBank databases under the accession number AY029283.

Abbreviations: LPS, lipopolysaccharide; EAE, experimental allergic encephalomyelitis; MAPK, mitogen-activated protein kinase; ERK, extracellular signal-regulated kinase; JNK/SAPK, c-Jun N-terminal kinase/stress-activated protein kinase; CNS, central nervous system; iNOS, inducible nitric oxide synthase

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kinase/stress-activated protein kinase (JNK/SAPK) [15]. These kinases are activated by phosphorylation of both tyrosine and threonine residues that is catalyzed by specific upstream MAPK kinase. Activated MAPKs phosphorylate their specific substrates on serine and/or threonine residues ultimately leading to activation of specific subsets of transcription factors. The p38 MAPK and JNK/SAPK are activated by inflammatory stimuli and environmental stress, while ERK is stimulated mainly by growth factors and tumor promoters. In the central nervous system (CNS), ERK and p38 MAPK have been shown to regulate inducible nitric oxide synthase (iNOS) and tumor necrosis factor-α (TNFα) gene expression in endotoxin-stimulated primary glial cultures [11]. In C6 glioma cells, however, iNOS expression was mediated by p38 MAPK, but not ERK [13].

Our understanding of physiological functions of caspase-11 and its evolutionary importance could be enhanced by identification of caspase-11 homologs in other species. It is of particular interest to identify the rat homolog of caspase-11, and to determine how its expression is regulated. This is because rat cerebral ischemia models are frequently used to investigate CNS inflammation and apoptosis, and up-regulation of certain caspases including caspase-11 has been reported to play a pathogenic role in these animal models [7,9]. Thus, the cloning of the rat homolog of caspase-11 is the prerequisite for studies on the role of this caspase in CNS using rat in vivo models. Based on previous reports on the critical functions of caspase-11 in CNS pathology [6–8] and the role of astrocytes in CNS inflammation [16], we hypothesized that the rat homolog of caspase-11 may be expressed in astrocytes, and its expression may be up-regulated during inflammatory activation of astrocytes. In the current work, we cloned rat caspase-11 cDNA and demonstrated that inflammatory induction of caspase-11 is mediated through the p38 subgroup of MAPK in rat astrocytes. Cloned rat caspase-11 cDNA can be further utilized to investigate physiological or pathological roles of caspase-11 in CNS inflammation and apoptosis, and to understand how astrocytes participate in these processes.

2. Materials and methods

2.1. Reagents and cells

LPS was obtained from Sigma (St. Louis, MO, USA). SB203580 and PD98059 were from Calbiochem (San Diego, CA, USA). Recombinant mouse TNFα, which has been shown to be active on rat C6 cells [17], and recombinant rat interferon-γ (IFNγ) were purchased from R&D Systems (Minneapolis, MN, USA). C6 rat glial cells were obtained from American Type Culture Collection (Manassas, VA, USA). The cell line was maintained in Dulbecco's modified Eagle's medium supplemented with 10% FBS, 2 mM glutamine, and penicillin–streptomycin (Gibco-BRL, Gaithersburg, MD, USA). Rat primary astrocytes were prepared as previously described with minor modifications [18,19]. In brief, forebrains of newborn Sprague–Dawley rats were chopped and dissociated by trypsinization and mechanical disruption. The cells were seeded into poly-L-lysine-coated culture flasks. After in vitro culture for 10 days, astrocytes were isolated by shaking of the culture flasks and reseeded into multi-well plates for assays. The purity of astrocyte cultures was greater than 90%, as determined by glial fibrillary acidic protein immunofluorescence staining (data not shown).

2.2. Cloning of rat caspase-11 cDNA and sequence analysis

Total RNA isolated from C6 cells was subjected to reverse transcription (RT) to obtain first strand cDNA. Degenerate oligonucleotide primers based on amino acid sequences of amino- and carboxy-terminal of mouse caspase-11 polypeptide were synthesized (Gibco-BRL) and used for amplification of rat caspase-11 cDNA from C6 cell cDNA by polymerase chain reaction (PCR) (annealing at 50°C with 35 cycles repeated). The nucleotide sequences of degenerate primers were: forward, ATG GCN GAR AAY AAR CAY CCN G; reverse, YYA RTT NCC NGG RAA NAG RTA RAA RTA YC (where N = A, C, G, or T; R = A or G; Y = C or T). PCR product was cloned into pCRII vector using a TOPO TA cloning kit (Invitrogen, Groningen, The Netherlands), and sequenced with T7 primer using an automated sequencing system (ABI 377; PE Applied Biosystems, Foster City, CA, USA). Sequence analyses were done using the GeneTool and PepTool program (DoubleTwist, Inc., Oakland, CA, USA)

2.3. In vitro transcription and translation

Coupled in vitro transcription and translation was performed as previously described [20]. The pCRII vector harboring rat caspase-11 cDNA was linearized by digestion with *Kpn*I. Linearized cDNA was gel-purified and used as a template in the coupled transcription and translation reaction using T7 RNA polymerase, [^{35}S]methionine, and TNT Coupled Reticulocyte Lysate Systems (Promega, Madison, WI, USA). Translation products were separated by 12% SDS–PAGE. The gel was dried and exposed to X-ray film for the analysis of translated polypeptides.

2.4. RT-PCR

Total RNA was extracted from C6 cells or primary astrocytes by a sequential addition of 4 M guanidinium thiocyanate, 2 M sodium acetate, and acid phenol/chloroform. RT was carried out using Superscript (Gibco-BRL) and oligo(dT) primer. PCR amplification using primer sets specific for each gene was carried out at 60°C annealing temperature for 30 cycles. Nucleotide sequences of the primers were based on the newly cloned rat caspase-11 or published cDNA sequences of caspase-1 and D-glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (caspase-11 forward, CTT CAC AGT GCG AAA GAA CTG; caspase-11 reverse, GAG TCC ACA TTA AGA AAT GTC TGG AGA AGC ATT TTG; caspase-1 forward, AAG ATG GCA CAT TTC CAG GAC; caspase-1 reverse, GGG CAC TTC AAA GTG TTC ATC; GAPDH forward, CTG CCA CTC AGA AGA CTG TGG; GAPDH reverse, CTT GAT GTC ATC ATA CTT GGC). Amplified RT-PCR products were separated by 1.5% agarose gel electrophoresis and visualized by ethidium bromide staining. The expected size of RT-PCR products is 275 bp for caspase-11, 447 bp for caspase-1 and 234 bp for GAPDH, respectively. The sequence of RT-PCR product was confirmed by direct sequencing. After an electrophoresis of PCR products on 1.5% agarose gel, the bands were excised and purified using the Geneclean kit (BIO 101 Inc., La Jolla, CA, USA). Purified PCR products were directly sequenced using an automated sequencing kit (ABI 377) and PCR primers.

2.5. Western blot analysis

Cells were lysed in triple-detergent lysis buffer (50 mM Tris–HCl, pH 8.0, 150 mM NaCl, 0.02% sodium azide, 0.1% SDS, 1% NP-40, 0.5% sodium deoxycholate, 1 mM phenylmethylsulfonyl fluoride). Protein concentration in cell lysates was determined using Bio-Rad protein assay kit. An equal amount of protein for each sample was separated by 12% SDS–PAGE and transferred to Hybond ECL nitrocellulose membrane (Amersham Pharmacia Biotech, Buckinghamshire, UK). The membrane was blocked with 5% skim milk and sequentially incubated with rabbit polyclonal antibodies specific for mouse/rat caspase-11 (R&D Systems), phosphorylated p38 MAPK (New England Biolabs, Beverly, MA, USA), phosphorylated p44/p42 MAPK (ERK 1/2) (New England Biolabs), or mouse monoclonal antibody against β-tubulin (Sigma), and then horseradish peroxidase-conjugated anti-rabbit or mouse antibodies (Amersham Pharmacia Biotech) followed by ECL detection (Amersham Pharmacia Biotech).

3. Results

3.1. Cloning of rat caspase-11 cDNA

To isolate rat cDNA encoding caspase-11, total RNA isolated from LPS-stimulated C6 rat glioma cells was subjected to RT-PCR using degenerate primers specific for the 5′ and 3′ ends of the coding region of mouse caspase-11. Primer sequences were based on published amino acid sequence of

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Consensus  MAEN-HPDKPLKVLE-LGKE-LTEYLEKLVQSNVLKLKEE-KQKFNNAER
rCasp-11   MAENNHPDKPLKVLEELGKEFLTEYLEKLVQSNVLKLKEEEKQKFNNAER   50
mCasp-11   MAENKHPDKPLKVLEQLGKEVLTEYLEKLVQSNVLKLKEEDKQKFNNAER   50

Consensus  SDKRWVFVD--K-KH--VG-MLLQTF--VD-GSHHGEANLEMEEPEES--
rCasp-11   SDKRWVFVDVLKRKHNQVGKMLLQTFLNVDSGSHHGEANLEMEEPEESVD  100
mCasp-11   SDKRWVFVDAMKKKHSKVGEMLLQTFFSVDPGSHHGEANLEMEEPEESLN  100

Consensus  TLKLCS-EEFTRLCREK-QEIYPIKE-NGRTRKALIICNTEFKHLSLRYG
rCasp-11   TLKLCSSEEFTRLCREKKQEIYPIKETNGRTRKALIICNTEFKHLSLRYG  150
mCasp-11   TLKLCSPEEFTRLCREKTQEIYPIKEANGRTRKALIICNTEFKHLSLRYG  150

Consensus  A--DI-GMKGLLE-LGYDVVVKEELTAEGMESEMKDFAALSEHQTSDSTF
rCasp-11   ANIDISGMKGLLEELGYDVVVKEELTAEGMESEMKDFAALSEHQTSDSTF  200
mCasp-11   AKFDIIGMKGLLEDLGYDVVVKEELTAEGMESEMKDFAALSEHQTSDSTF  200

Consensus  LV-MSHGTL-G-CGTMHSE-TPDVL-YDTIYQIFNNCHCPGLRDKPKVII
rCasp-11   LVVMSHGTLQGLCGTMHSEATPDVLLYDTIYQIFNNCHCPGLRDKPKVII  250
mCasp-11   LVLMSHGTLHGICGTMHSEKTPDVLQYDTIYQIFNNCHCPGLRDKPKVII  250

Consensus  VQACRGGNSGE-WIRESS-----R-VDLPRNMEADAV--SHVEKDFIA-Y
rCasp-11   VQACRGGNSGEVWIRESSGAHSYRAVDLPRNMEADAVRMSHVEKDFIALY  300
mCasp-11   VQACRGGNSGEMWIRESSKPQLCRGVDLPRNMEADAVKLSHVEKDFIAFY  300

Consensus  STTPHHLSYRDKT-GSYFI--LISCFR-HACSCHLFDIFLKVQQSFEKAS
rCasp-11   STTPHHLSYRDKTRGSYFISKLISCFRIHACSCHLFDIFLKVQQSFEKAS  350
mCasp-11   STTPHHLSYRDKTGGSYFITRLISCFRKHACSCHLFDIFLKVQQSFEKAS  350

Consensus  I-SQMPTIDRATLTRYFYLFPGN
rCasp-11   INSQMPTIDRATLTRYFYLFPGN                            373
mCasp-11   IHSQMPTIDRATLTRYFYLFPGN                            373
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Fig. 1. Amino acid sequence alignment of rat and mouse caspase-11. Nucleotide sequence of cloned rat caspase-11 (rCasp-11) cDNA was determined and its deduced amino acid sequence was compared with mouse caspase-11 (mCasp-11). Predicted Asp (D) residues of cleavage sites and pentapeptide sequence 'QACRG' that contains the catalytic cysteine residue are underlined.

mouse caspase-11 protein. Amplified RT-PCR products were cloned and sequenced. Deduced amino acid sequence of rat caspase-11 protein showed 88.7% sequence homology to mouse caspase-11 (Fig. 1). Rat caspase-11 also showed 59.5% and 53.9% amino acid sequence similarity to human caspases-4 and -5, respectively, which are candidates for human homolog of caspase-11. Predicted Asp residues of cleavage sites and pentapeptide sequence 'QACRG' that contain the catalytic cysteine residues were well conserved between mouse and rat caspase-11. In vitro transcription and translation of rat caspase-11 cDNA yielded a polypeptide of approximately 43 kDa, which was in agreement with predicted protein size generated from full-length rat caspase-11 cDNA (Fig. 2).

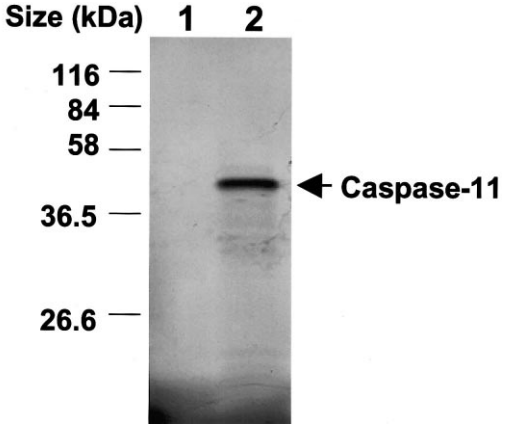

Fig. 2. In vitro translation of rat caspase-11. Caspase-11 was in vitro translated and analyzed by 12% SDS–PAGE, followed by autoradiography (lane 2). As a control, in vitro transcription and translation were also done without caspase-11 cDNA (lane 1). The arrow indicates presumed rat caspase-11 protein of ~43 kDa.

3.2. Induction of caspase-11 by inflammatory stimuli in astrocytes

Previous studies demonstrated that the expression of mouse caspase-11 in thymus, lung, and spleen is induced by LPS injection [2]. Recently, it has been also reported that caspase-11 expression in brain is up-regulated following focal ischemia [9] and EAE [6]. Thus, we next analyzed inducibility of rat caspase-11 expression in astrocytes after treatment with inflammatory stimuli. LPS, IFNγ, and TNFα all induced the expression of caspase-11 mRNA in C6 glial cells (Fig. 3A). A similar pattern of caspase-11 induction at the protein level was

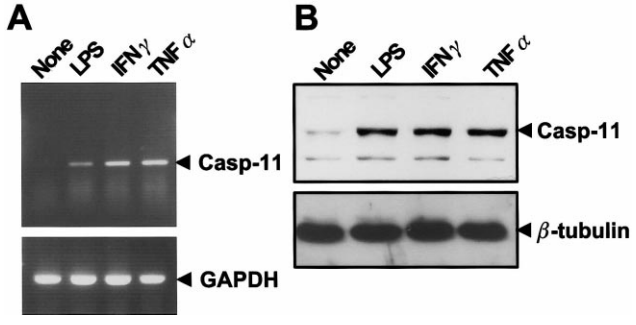

Fig. 3. Induction of caspase-11 by inflammatory stimuli in C6 cells. C6 cells were treated with LPS (100 ng/ml), IFNγ (100 U/ml), or TNFα (10 ng/ml) for 24 h, and then the levels of caspase-11 messages or protein were evaluated by RT-PCR (A) or Western blot analysis (B). Amounts of GAPDH or β-tubulin were also assessed to confirm the integrity and equal loading of the samples. LPS, IFNγ, and TNFα all induced caspase-11 expression. Results are the representative of three independent experiments.

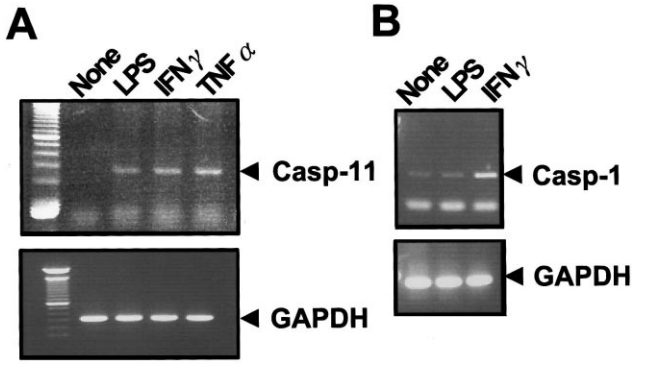

Fig. 4. Induction of caspase-11 in primary astrocyte cultures. Rat primary astrocytes were treated with LPS (100 ng/ml), IFNγ (100 U/ml), or TNFα (10 ng/ml) for 24 h, and then caspase-11 or caspase-1 messages were analyzed by RT-PCR. While caspase-11 was induced by LPS, IFNγ, and TNFα, caspase-1 was induced only by IFNγ. Rat GAPDH was used as an internal control in RT-PCR. The amplified products were not observed in RT-PCR without reverse transcriptase (data not shown). Results are the representative of three independent experiments.

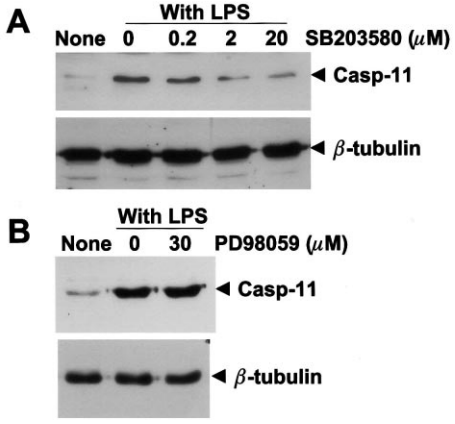

Fig. 6. Induction of caspase-11 by LPS through p38 MAPK. C6 cells were pretreated with increasing concentrations of SB203580 (0.2–20 μM) or PD98059 (30 μM) for 1 h before LPS treatment for 24 h. Then, levels of caspase-11 protein were assessed by Western blot analysis. β-Tubulin was also analyzed to show the equal loading of samples in each lane. Results are the representative of three independent experiments.

confirmed by Western blot analysis (Fig. 3B). Induction of caspase-11 messages by inflammatory stimuli was also observed in rat primary astrocytes (Fig. 4A). Caspase-1 messages were also analyzed for comparison. A low level of caspase-1 was constitutively expressed in astrocytes and its expression was induced by IFNγ, but not by LPS (Fig. 4B), suggesting partly different regulatory mechanisms between caspase-11 and caspase-1 expressions.

3.3. LPS induction of caspase-11 through p38 MAPK

In order to determine whether inflammatory induction of caspase-11 expression is mediated through MAPK pathways, we analyzed activation of two subgroups of MAPK pathways, p38 kinase and ERK, in C6 cells stimulated with LPS. LPS strongly induced activation of p38 MAPK as demonstrated by the appearance of phosphorylated form of the kinase (Fig. 5). In contrast, phosphorylated ERK protein was detected in resting cells, and the intensity of the protein band was slightly

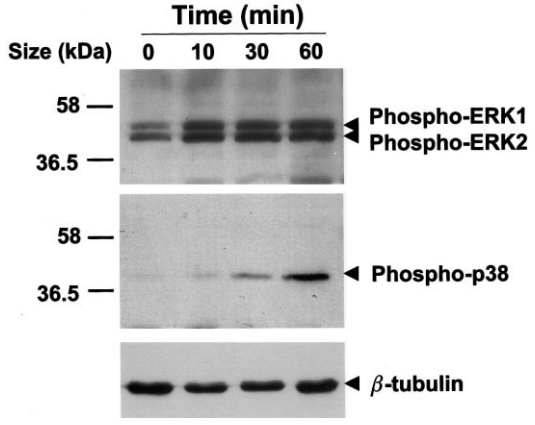

Fig. 5. Activation of p38 MAPK and ERK by LPS. C6 cells were treated with LPS (100 ng/ml) for the indicated time periods, and activation of ERK and p38 MAPK was assessed by Western blot analysis using antibodies specific for phosphorylated ERK or p38 MAPK. Anti-β-tubulin antibody was also employed to show the equal loading of samples in each lane. Results are the representative of four independent experiments.

increased in response to LPS (Fig. 5). We next employed specific chemical inhibitors of p38 MAPK or ERK pathways. Pretreatment of C6 cells with SB203580 (a specific inhibitor of p38 kinase) before LPS treatment dose-dependently inhibited the induction of caspase-11 protein (Fig. 6A). However, 30 μM of PD98059 (a specific inhibitor for ERK kinase, MEK) did not affect caspase-11 induction (Fig. 6B). Inability of PD98059 to inhibit caspase-11 induction was not due to the dose of the inhibitor used. Higher concentrations of PD98059 were also without effects (data not shown). Moreover, 25 μM of PD98059 has been previously shown to inhibit ERK pathways and subsequent iNOS induction in rat astrocytes [11]. These results indicated that LPS-stimulated caspase-11 induction in C6 cells was mediated through p38 MAPK pathway, but not ERK pathway.

4. Discussion

In this work, we molecularly cloned rat caspase-11 cDNA and presented evidence that caspase-11 is induced by inflammatory signals through p38 MAPK pathway in astrocytes. Since the first cloning of mouse caspase-11, a caspase-11 homolog in any other species has not been identified. Caspase-4 and -5 have been proposed as the possible human homolog of caspase-11 [4]. Among known caspase family members, caspase-4 showed the strongest sequence homology to mouse caspase-11, however, its inflammatory inducibility was not observed. Recently, caspase-5 has been shown to be markedly induced by LPS in the THP-1 human monocytic cell line [5]. Thus, in spite of relatively weak sequence homology compared to caspase-4, caspase-5 may be the human homolog of caspase-11. Here, we identified for the first time an authentic homolog of caspase-11 in rat. Our work demonstrated that amino acid sequence and inducibility of caspase-11 were well conserved between mouse and rat. Sequence analysis showed that rat caspase-11 has higher sequence similarity to human caspase-4 than to caspase-5. Our current cloning of rat caspase-11 and future identification of caspase-11 homologs in other species will not only help identify the real human homolog of the caspase, but also enhance our understanding of

physiological functions and evolutionary importance of this caspase.

Previous works in our laboratory and others on the role of caspase-11 in CNS pathology [6–8] and inflammatory induction of the caspase-11 in microglia [10] led us to examine the inducibility of this caspase in astrocytes and signal transduction pathways involved. Astrocytes play a central role in CNS inflammation. Activated astrocytes have been shown to produce a variety of inflammatory mediators, which include reactive oxygen species and proinflammatory cytokines [16]. In particular, IL-1 is secreted by human and rat astrocyte cultures [21,22]. Moreover, we have previously shown that mouse astrocytes also produce IL-18 [23]. Proforms of IL-1 and IL-18 require protease-mediated processing in order to be secreted. Caspase-1 is the very protease responsible for such processing [24], and caspase-1 in turn requires caspase-11 for its activation. Thus, the expression of caspase-11 is vital for the production of these cytokines in astrocytes. Our current work demonstrated that caspase-11 is strongly induced by inflammatory stimuli such as LPS, IFNγ, and TNFα in astrocytes. These results suggest that the presence of an inflammatory amplification mechanism in the CNS, whereby astrocytes activated by inflammatory stimuli may produce yet other proinflammatory cytokines. Inflammatory induction of caspase-11 in astrocytes has an additional meaning besides positive amplification of inflammatory responses involving astrocytes. Caspase-11 has been proposed as an initiator caspase that can be auto-activated when its intracellular expression is elevated [7]. Auto-activated caspase-11 initiated caspase cascade, activating caspase-1 or -3 [7]. Thus, caspase-11 induction in astrocytes may potentially result in activation of effector caspases (caspase-1 or -3), leading to apoptosis of astrocytes. We have previously shown that rat astrocytes and microglial cells undergo auto-regulatory apoptosis upon inflammatory activation [19,25]. Although NO was the major apoptotic mediator in microglia, the induction of caspase-11 by inflammatory stimulation constituted the NO-independent apoptotic pathway in activation-induced cell death of microglia [10]. In astrocytes, inflammatory induction of caspase-11 may well play a similar role.

Previously, induction of caspase-11 has been observed after focal ischemia in mouse and rat middle cerebral artery occlusion models as well as in the EAE model [6,7,9]. However, the expression of caspase-11 was detected only in neurons, microglia, and oligodendrocytes. To our knowledge, our results are the first to report on the expression of caspase-11 in astrocytes. We demonstrated the induction of caspase-11 both in C6 glial cells and primary astrocyte cultures. Results from C6 cells and primary astrocytes complemented each other. Experimental use of primary astrocyte cultures is generally preferred compared to established cell lines to avoid undesirable modifications of cellular characteristics resulting from long-term cultures or cellular transformation. The use of primary astrocytes, however, raises a concern about possible contamination of a small number of microglial cells. This may be one of the limitations of using primary cells. Our results obtained from homogeneous C6 cell lines complemented the results from primary cells, supporting that astrocytes indeed express caspase-11 when stimulated.

The p38 MAPK has been shown to be involved in astrocyte gene expression [11–14]. A canonical example is iNOS gene expression. The p38 MAPK pathway was specifically involved in TNFα/IL-1α-induced iNOS gene expression in mouse astrocyte cultures [12] and in LPS-induced expression of the gene in rat astrocytes [11]. The p38 MAPK, but not p44/p42 MAPK (ERK), mediated LPS/IFNγ/TNFα-induced iNOS expression in C6 rat glioma cells [13]. However, there are reports from other laboratories that are in contrast to these results. In glomerular mesangial cells, activation of p38 MAPK mediated the down-regulation of iNOS expression induced by IL-1β [26]. Also, in C6 cells, transcriptional activation of the iNOS gene by IFNγ was independent of Ras–MAPK pathway [27]. Thus, the role of MAPK pathways in induction of iNOS gene expression appears to depend on cell types and stimuli investigated. Moreover, even in the same cell types, the same stimuli may use diverse signaling pathways for the induction of different sets of target genes. Regulation of caspase-11 expression may well be as complex as iNOS. As presented in the present studies, p38 MAPK mediated LPS-induced caspase-11 expression in C6 cells. However, as more studies are conducted in the future, it is possible that results on the role of p38 MAPK in caspase-11 expression may be different depending on cell types, stimuli, and species tested.

In conclusion, we have shown in this work that caspase-11 expression is strongly induced by inflammatory signals in rat astrocytes, and p38 MAPK pathway may be involved in these processes. Inflammatory induction of caspase-11 in astrocytes may play an important role in both inflammatory responses involving these cells and auto-regulatory apoptosis of activated astrocytes in inflammatory sites.

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