Interleukin-1β Induces Chronic Activation and de Novo Synthesis of Neutral Ceramidase in Renal Mesangial Cells*

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The lipid signaling molecule ceramide is formed by the action of acid and neutral sphingomyelinases and degraded by acid and neutral ceramidases. Short-term stimulation of mesangial cells with the pro-inflammatory cytokine interleukin-1β (IL-1β) leads to a rapid and transient increase in neutral sphingomyelinase activity (Kaszkin, M., Huwiler, A., Scholz, K., van den Bosch, H., and Pfeilschifter, J. (1998) FEBS Lett. 440, 163–166). In this study, we report on a second delayed peak of activation occurring after hours of IL-1β treatment. This second phase of activation was first detectable after 2 h of treatment and steadily increased over the next 2 h, reaching maximal values after 4 h. In parallel, a pronounced increase in neutral ceramidase activity was observed, accounting for a constant or even decreased level of ceramide after long-term IL-1β treatment, despite continuous sphingomyelinase activation. The increase in neutral ceramidase activity was due to expressionional up-regulation, as detected by an increase in mRNA levels and enhanced de novo protein synthesis. The increase in neutral ceramidase protein levels and activity could be blocked dose-dependently by the p38 MAPK inhibitor SB 202190, whereas the classical MAPK pathway inhibitor U0126 and the protein kinase C inhibitor Ro 318220 were ineffective. Moreover, cotreatment of cells for 24 h with IL-1β and SB 202190 led to an increase in ceramide formation. Interestingly, IL-1β-stimulated neutral ceramidase activation was not reduced in mesangial cells isolated from mice deficient in MAPK-activated protein kinase-2, which is a downstream substrate of p38 MAPK, thus suggesting that the p38 MAPK-mediated induction of neutral ceramidase occurs independently of the MAPK-activated protein kinase-2 pathway. In summary, our results suggest a biphasic regulation of sphingomyelin hydrolysis in cytokine-treated mesangial cells with delayed de novo synthesis of neutral ceramidase counteracting sphingomyelinase activity and apoptosis. Neutral ceramidase may thus represent a novel cytoprotective enzyme for mesangial cells exposed to inflammatory stress conditions.

The mesangial cell is a smooth muscle cell-like pericyte located in the renal glomerulus and is a key player in the glomerular inflammatory response (1–3). Inflammatory diseases of the renal glomerulus are accompanied by enhanced formation of the pro-inflammatory cytokine interleukin-1β (IL-1β).1 The primary source is the activated macrophage, but IL-1β is also released by many other cell types after exposure to an inflammatory environment. Soluble IL-1β is the predominant form in biological fluids, and it binds to specific receptors in target tissues. IL-1 is an exemplary pro-inflammatory cytokine that is particularly important in the systemic response to inflammation. It synergizes with tumor necrosis factor-α (TNF-α) for many of its actions, and its synthesis is stimulated, in turn, by TNF-α. Furthermore, it is implicated in the pathogenesis of diseases such as rheumatoid arthritis, inflammatory bowel disease, septic shock, and several autoimmune reactions.

In the past, it has become clear that sphingolipids exert important roles as signaling molecules under various physiological and pathophysiological conditions (4–7). Especially ceramide has attracted a lot of interest due to its potential involvement in regulation of programmed cell death, cell growth arrest, and differentiation (4–7). However, the regulating mechanisms that determine the intracellular ceramide level are still poorly understood. Most studies have focused on the ceramide-generating enzymes, i.e. the acid and neutral sphingomyelinases. Based on activity measurements from cell extracts, activators of acid and/or neutral sphingomyelinases have been determined and include pro-inflammatory cytokines, growth factors, and other environmental stress stimuli (4, 7). However, sphingomyelinases depict only one side of the regulation of ceramide levels. It is equally important to understand the involvement of ceramide-degrading enzymes, the ceramidas, which hydrolyze ceramide to yield sphingosine. Sphingosine on its own can act either in a proliferative (8–10) or pro-apoptotic (11–14) manner depending on the cell system, but it can also serve as a substrate for sphingosine kinase to yield sphingosine 1-phosphate (8), which is a potent mitogen for several cell types (15, 16). Due to this equally important role of ceramidas in determining cellular levels of ceramide, which is a pro-apoptotic stimulus, and sphingosine 1-phosphate being a proliferative stimulus, it is essential to understand the regulation of these enzymes.

It has become clear that there are at least two subtypes of ceramidas existing in mammalian cells: an acidic form, which is localized in the lysosomes (17), the main organelle involved in lipid degradation, and a neutral/alkaline form.

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1 The abbreviations used are: IL-1β, interleukin-1β; TNF-α, tumor necrosis factor-α; PAGE, polyacrylamide gel electrophoresis; PCR, polymerase chain reaction; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; bp, base pair; MAPK, mitogen-activated protein kinase; MAPKAPK-2, mitogen-activated protein kinase-activated protein kinase-2; ERK, extracellular signal-regulated kinase; MEK, mitogen-activated protein kinase/extracellular signal-regulated kinase.
(18, 19), which has only recently been cloned and about which not much is known regarding its localization or activation. It is tempting to speculate that this enzyme plays an equally important role in signal transduction as the sphingomyelinase and it counterbalances ceramide generation by the latter enzyme. Biochemical characterization of this novel neutral ceramidase reveals that it is a 94-kDa enzyme in mouse tissue (18) and a 110–120-kDa enzyme in rat tissue (19), containing several putative protein kinase C and casein kinase II phosphorylation sites in its primary sequence. Recently, El Bawab et al. (20) cloned another human neutral ceramidase that contained an N-terminal mitochondrial signal peptide and therefore was suggested to be a mitochondrial enzyme.

In this study, we investigated the effect of the pro-inflammatory cytokine IL-1β on the neutral sphingomyelinase and neutral ceramidase activities in rat mesangial cells. We show that chronic IL-1β treatment of mesangial cells leads to a biphasic activation of the neutral sphingomyelinase and a delayed activation of the neutral ceramidase, ultimately leaving cellular ceramide levels low. The activation of the neutral ceramidase is due to expressional up-regulation of the gene.

**EXPERIMENTAL PROCEDURES**

**Chemicals**—[1-14C]Serine (specific activity, 55 Ci/mol), [1-14C]sphingomyelin (specific activity, 55 Ci/mol), [14]Smethionine and [35]Systeine pro-mix (specific activity, >1000 Ci/mmol), [α-32P]dCTP (specific activity, 3000 Ci/mmol), and protein A-Sepharose CL-4B were from Amersham Pharmacia Biotech (Freiburg, Germany). [1-14C]Ceramide (specific activity, 55 Ci/mol) was from ICN Biomedicals GmbH (Eschwege, Germany). SB 202190, U0126 and Ro 318220 were from Biomol (Bonn, Germany). [35]S]methionine and [35]Systeine were added (140 μCi/plate). After labeling, cells were washed twice with ice-cold phosphate-buffered saline and then scraped directly into 1 ml of lysis buffer and homogenized. The homogenate was centrifuged for 10 min at 14,000 × g, and the supernatant was taken for immunoprecipitation. Samples of 1-ml volume containing 250 × 10^{6} cpm of labeled proteins, 5% fetal calf serum, and 1.5 mCi iodoacetamide in lysis buffer were incubated overnight at 4°C with anti-ceramide- and neutral ceramidase-specific antibodies. The precipitate was washed twice with low salt buffer (50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 0.2% Triton X-100, 2 mM EDTA, 2 mM EGTA, and 0.1% SDS), three times with high salt buffer (50 mM Tris-HCl (pH 7.5), 500 mM NaCl), 0.2% Triton X-100, 2 mM EDTA, 2 mM EGTA, and 0.1% SDS), and once with 10 mM Tris. Pellets were boiled for 5 min at 100°C and subjected to SDS-PAGE. After fixing in 25% isopropyl alcohol and 10% acetic acid, the gels were dried and exposed on a Bio-Imaging Analyzer (Fuji).

**Reverse Transcription-PCR**—Total RNA was isolated using guanidinium isothiocyanate isolation. 1.5 μg of RNA were used for reverse transcription-PCR (first strand cDNA synthesis kit, MBI Fermentas, St. Leon-Rot, Germany). PCR was carried out as follows (Taq polymerase, recombinant, MBI Fermentas, St. Leon-Rot, Germany): 94°C for 5 min (one cycle); 94°C for 1 min, 52°C for 1.5 min, and 72°C for 1 min (with a variable number of cycles); and a final extension at 72°C for 7 min. The number of cycles was 30 for murine neutral ceramidase, 45 for rat neutral ceramidase, and 25 for glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The sequences of the primers for analysis of mRNA were as follows: mouse neutral ceramidase, TTC AAT GCC GGT GAT CTA C (forward) and GCC AGG AAT CAC ACA AA (reverse); rat neutral ceramidase, AGA AGA GCT GTA AAG CCG C (forward) and TGC GAT AAT GAC AGT CAT ATC C (reverse); and GAPDH, AAT GCA TGC TGC ACC ACC AA (forward) and GTC ATT GAC GAG AAT GCC GGC C (reverse). PCR products (785-bp length for mouse neutral ceramidase, 377-bp length for rat neutral ceramidase, and 470-bp length for GAPDH) were run on a 1.5% agarose gel containing 0.5 μg/ml ethidium bromide. The identities of amplicons were confirmed by sequencing using a Model 310 genetic analyzer (PerkinElmer Life Sciences).

**Western Blot Analysis**—Total RNA was isolated using guanidinium isothiocyanate solution. 25 μg of RNA were separated by electrophoresis on formaldehyde-containing 1% agarose gels. RNA was transferred to a nylon membrane by vacuum blotting for 2 h at 50 millibars and cross-linked by UV light. Blots were hybridized with a 540-bp reverse transcriptase-PCR product (forward primer, CGA GGC GCA AGG AGC AA (forward) and GTC ATT GAC GAG AAT GCC GGC C (reverse)); PCR products (785-bp length for mouse neutral ceramidase). Blots were hybridized with a 540-bp reverse transcriptase-PCR product (forward primer, CGA GGC GCA AGG AGC AA (forward) and GTC ATT GAC GAG AAT GCC GGC C (reverse)); PCR products (785-bp length for mouse neutral ceramidase, 377-bp length for rat neutral ceramidase, and 470-bp length for GAPDH) were run on a 1.5% agarose gel containing 0.5 μg/ml ethidium bromide. The identities of amplicons were confirmed by sequencing using a Model 310 genetic analyzer (PerkinElmer Life Sciences).

**Metabolic Labeling of Cells and Immunoprecipitation**—Confluent mesangial cells in 100-mm diameter dishes were washed with phosphate-buffered saline and incubated in methionine-free Dulbecco's modified Eagle's medium in the absence or presence of the stimulators for the indicated time periods. For the last 4 h of incubation, [35]Smethionine and [35]Systeine were added (140 μCi/plate). After labeling, cells were washed twice with ice-cold phosphate-buffered saline and then scraped directly into 1 ml of lysis buffer and homogenized. The homogenate was centrifuged for 10 min at 14,000 × g, and the supernatant was taken for immunoprecipitation. Samples of 1-ml volume containing 250 × 10^{6} cpm of labeled proteins, 5% fetal calf serum, and 1.5 mCi iodoacetamide in lysis buffer were incubated overnight at 4°C with anti-ceramide- and neutral ceramidase-specific antibodies. The precipitate was washed twice with low salt buffer (50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 0.2% Triton X-100, 2 mM EDTA, 2 mM EGTA, and 0.1% SDS), three times with high salt buffer (50 mM Tris-HCl (pH 7.5), 500 mM NaCl), 0.2% Triton X-100, 2 mM EDTA, 2 mM EGTA, and 0.1% SDS), and once with 10 mM Tris. Pellets were boiled for 5 min at 100°C and subjected to SDS-PAGE. After fixing in 25% isopropyl alcohol and 10% acetic acid, the gels were dried and exposed on a Bio-Imaging Analyzer (Fuji).
isocratic mode with a Nucleosil C$_18$ column (30 × 2.0-mm inner diameter, 5-µm particle size, and 100-Å pore size; Macherey-Nagel, Düren, Germany). The mobile phase consisted of methanol containing 5 mM ammonium acetate. The flow rate was set at 0.2 ml/min. The injection volume was 10 µl, and the run time was 3 min. The turbo ion spray interface was operated in the positive ion mode at 5200 V and 200 °C and was supplied by an auxiliary gas flow of 4500 ml/min. The nebulizer gas was set at 1.23 liters/min (setting 10); the curtain gas flow was set at 1.08 liters/min (setting 9); and the collision gas was set at 3.7 × 10$^{-6}$ hectaropascals (3.02 × 10$^{-22}$ molecules/cm$^2$, setting 4). Nitrogen was used for all gases. C16:0 ceramide standards and cellular lipid extracts were resolved in 1000 µl of 5 mM ammonium acetate/methanol buffer just prior to mass spectrometric analysis. Standards were analyzed at concentrations ranging from 25 nM to 10 µM.

Quantitation was performed by multiple reaction monitoring (dwell time, 200 ms) of the protonated precursor ion and related product ions. The mass transition used for quantification was m/z 538.4 → 264.2 (collision energy, 33 eV). The mass transitions used as qualifier were m/z 538.4 → 292.1 (collision energy, 39 eV). The analytical data were processed by Analyst software (Version 1.1).

**Acid and Neutral Sphingomyelinase Activity Assays**—Confluent mesangial cells in 60-mm diameter dishes were incubated with the indicated stimuli in Dulbecco’s modified Eagle’s medium containing 0.1 mg/ml fatty acid-free bovine serum albumin for the indicated time periods. Neutral and acid sphingomyelinase activities were measured according to Liu and Hannun (24) and Quinlter et al. (25) with some modifications as previously described (26).

**Acid and Neutral Ceramidase Activity Assays**—Confluent mesangial cells were stimulated as described above and homogenized in lysis buffer containing 50 mM sodium acetate (pH 4.5), 0.5% Triton X-100, 5 mM MgCl$_2$, 1 mM EDTA, and 5 mM β-galactonic acid γ-lactone for the acid ceramidase and 50 mM Tris (pH 7.5), 0.5% Triton X-100, 5 mM MgCl$_2$, 1 mM EDTA, and 5 mM β-galactonic acid γ-lactone for the neutral ceramidase. Activity assays were performed according to Mitutake et al. (27) with some modifications as previously described (26).

**Statistical Analysis**—Statistical analysis was performed by one-way analysis of variance. For multiple comparisons with the same control group, the limit of significance was divided by the number of comparisons according to Bonferroni.

**RESULTS**

**IL-1β Stimulates Chronic Activation of Neutral Sphingomyelinase**—Previously, we have shown that the pro-inflammatory cytokine IL-1β causes a rapid (within minutes) and transient activation of neutral sphingomyelinase activity in rat mesangial cells (28), which leads to increased ceramide formation (28, 29). We now have extended these studies and found that prolonged treatment of mesangial cells with IL-1β resulted in a delayed second peak of neutral sphingomyelinase activation that was first detectable after 2 h of stimulation and reached a maximum after 4 h (Fig. 1A). The acid sphingomyelinase also showed a time-dependent delayed activation after IL-1β treatment (Fig. 1B).

Surprisingly, when the level of ceramide was measured by tandem mass spectrometry after IL-1β stimulation, no increase was observed up to 24 h after stimulation (Fig. 1C), thus pointing toward additional compensatory mechanisms that regulate the ceramide content of the cell. In contrast, 1 h of stimulation with a bacterial sphingomyelinase led to an 8–10-fold increase in ceramide levels (Fig. 1C).

**IL-1β Stimulates Chronic Activation of a Neutral Ceramidase**—To investigate the effect of IL-1β on the ceramide-degrading enzymes, rat mesangial cells were stimulated for different time periods with the cytokine, and ceramidase activity was measured. As shown in Fig. 2 (A and B), IL-1β caused a chronic activation of acid and neutral ceramidases, with maximal stimulation occurring 4 h after cytokine exposure and subsequently remaining at high levels.

**IL-1β Stimulation Leads to Neutral Ceramidase mRNA and Protein Up-regulation**—To test whether the increase in neutral ceramidase activity is due to an increased amount of neutral ceramidase protein, Western blot analysis was performed using a polyclonal antiserum raised against a peptide comprising the N terminus of the murine neutral ceramidase.

The antiserum recognized a double band of ~110–120 kDa. This size is in agreement with the recently described size of rat kidney neutral ceramidase (19). To determine whether the recognized protein does indeed show neutral ceramidase activity, cell lysates from IL-1β-stimulated mesangial cells were separated on a MonoQ column, and fractions were analyzed by Western blotting (Fig. 3A, upper panel) and for neutral ceramidase activity (lower panel). Earlier fractions (fractions 9 and 10) showed an ~94-kDa protein of still unknown identity that was recognized by the neutral ceramidase antibody. Fractions 11 and 12 showed exclusive expression of a 110–120-kDa protein, the predicted size of rat neutral ceramidase (19). The neutral ceramidase activity was highest in fractions 11 and 12, which also showed the highest protein amounts, thus suggesting that this band is indeed a neutral ceramidase. Furthermore, we investigated whether the antibody could immunoprecipitate a fully active enzyme. As shown in Fig. 3B, Western blot analysis of the supernatant after immunoprecipitation of neutral ceramidase revealed a disappearance of the protein that was dependent on the antibody dilution used (Fig. 3B, upper panel). Preimmune serum did not deplete the protein from the supernatant. In parallel, a reduction of neutral ceramidase activity was observed in the supernatant (Fig. 3B, lower panel). Consistent with a depletion of the enzyme in the supernatant, an increased amount of enzyme was observed in the immunoprecipitates by Western blotting (Fig. 3C). However, no increased neutral ceramidase activity was recovered in the immunoprecipitates (data not shown). These data suggest that binding of the antibody to its antigen leads to a neutralization of the enzyme activity.

Stimulation of mesangial cells with IL-1β led to a marked and time-dependent up-regulation of the neutral ceramidase protein (Fig. 4A). In contrast, the acid ceramidase protein, which runs at 55 kDa as a holoenzyme under nonreducing conditions (30), was not significantly changed (Fig. 4B).

We further investigated whether the up-regulation of neutral ceramidase is due to increased de novo synthesis. For this purpose, mesangial cells were stimulated with IL-1β for different time periods, and [35S]methionine and [35S]cysteine were included in the culture medium for the last 4 h of stimulation. Thereafter, the cells were lysed, and the neutral ceramidase was immunoprecipitated and analyzed by SDS-PAGE. Fig. 5 shows that IL-1β triggered increased de novo synthesis of the neutral ceramidase. A similar increase was also observed with another pro-inflammatory cytokine, TNF-α (Fig. 5). In contrast, the degradation of the neutral ceramidase was not affected by IL-1β treatment (data not shown) as analyzed by pulse-chase experiments.

In a next step, we tested whether there is also an induction of the mRNA coding for the neutral ceramidase. Based on the mouse sequence of neutral ceramidase, mouse primers were selected and used to obtain a cDNA for the rat sequence. Using this partial sequence of the rat neutral ceramidase, new primers were generated and used to perform reverse transcriptase-PCR of IL-1β-stimulated rat mesangial cells. IL-1β stimulation indeed led to a clear induction of the neutral ceramidase mRNA level (Fig. 6A). A maximal induction was obtained after 4 h of stimulation and slightly decreased over the next 20 h. A similar induction was obtained in mouse mesangial cells using mouse primers (data not shown). Furthermore, the induction of neutral ceramidase by IL-1β was confirmed by Northern blot analysis (Fig. 6B). Interestingly, two transcripts were detected for the neutral ceramidase at 3.5 kilobases, which were both induced by IL-1β (Fig. 6B, upper panel). In contrast, the acid ceramidase activity was not induced.
ceramidase mRNA was not significantly altered by IL-1β stimulation (Fig. 6B, lower panel).

**IL-1β-induced Up-regulation of Neutral Ceramidase Involves the p38 MAPK Pathway, but Not MAPKAPK-2**—To further elucidate mechanistically the pathway by which IL-1β increases neutral ceramidase activity, we tested inhibitors against the different MAPK cascades, i.e. the classical ERK and the stress-activated protein kinase p38 MAPK, since these MAPKs are known to play an important role in activating transcription factors and subsequently gene transcription and are targeted by rather specific low molecular mass inhibitors.

SB 202190, which is a quite selective inhibitor of p38 MAPK (31), caused a dose-dependent decrease in IL-1β-induced neutral ceramidase activity (Fig. 7A) as well as in protein induction (Fig. 7B). SB 202190 alone had no effect on ceramidase activity or protein levels (Fig. 7A and B). Consequently, we found that cotreatment of IL-1β with SB 202190, which blocks neutral ceramidase activity, but leaves the IL-1β-induced persistent sphingomyelinase activation unaffected, resulted in increased formation of ceramide (Fig. 7C). In parallel, an enhanced rate of apoptosis was seen under cotreatment conditions (data not shown). SB 202190 had no effect on IL-1β-stimulated neutral or acid sphingomyelinase or acid ceramidase activities (data not shown). In contrast to SB 202190, U0126, which inhibits the MAPK kinase MEK, and Ro 318220, which potently blocks protein kinase C isoenzymes, were ineffective in blocking neutral ceramidase activity (data not shown).

As MAPKAPK-2 is a downstream substrate of p38 MAPK, which can phosphorylate various transcription factors and thereby regulate gene transcription (32–34), we further investigated whether MAPKAPK-2 is involved in IL-1β-mediated neutral ceramidase activation. For this purpose, we isolated mesangial cells from MAPKAPK-2 knockout mice (33) and corresponding control mice and stimulated these cells with IL-1β. As shown in Fig. 8, IL-1β-induced neutral ceramidase activity was not abolished in these mice, thus suggesting that MAPKAPK-2 does not mediate p38 MAPK-triggered neutral ceramidase induction.

**DISCUSSION**

In this study, we have shown that IL-1β evokes a biphasic activation of neutral sphingomyelinase activity in renal mesan-
ceramide-generating and -degrading enzymes is a stable level of ceramide, which even tends to decrease over prolonged time periods of stimulation. Similar results regarding a balanced regulation of neutral ceramidase and neutral sphingomyelinase activities and ceramide levels in mesangial cells were also observed for TNF-α (26).

These findings are consistent with the data of Nikolova-Karakashian et al. (35), who found that, in rat hepatocytes, IL-1β also chronically increases neutral ceramidase activity and fails to accumulate ceramide in the cells. Additionally, these authors found that vanadate, a tyrosine phosphatase inhibitor, dramatically enhances IL-1-induced neutral ceramidase activity, whereas the nonspecific tyrosine kinase inhibitor genistein partially inhibits it. Whether this is due to phosphorylation and subsequent changes in enzyme activity or changes in the expression level of the enzyme were not addressed.

Furthermore, Coroneos et al. (36) reported that platelet-derived growth factor is a potent activator of ceramidase activity in rat mesangial cells, whereas cytokines such as IL-1β and TNF-α are ineffective in activating ceramidase after 1 h of stimulation. These data do not contrast with our results, as 1 h of IL-1β stimulation was not sufficient to increase the neutral ceramidase activity, and at least 2–4 h of stimulation were required to see significant stimulation of enzyme activity (Fig. 2A). Again, the short-term activation of neutral/alkaline ceramidase by platelet-derived growth factor observed by Coroneos et al. (36) was suggested to involve tyrosine kinases since the platelet-derived growth factor-induced activation was completely inhibited by genistein. Taken together, these reports and our own results make it tempting to speculate that the neutral ceramidase is regulated by two different mechanisms: (i) a rapid post-translational regulation by phosphorylation/phosphorylation reactions, which is further supported by the presence of various putative protein kinase phosphorylation sites in the sequence of the neutral ceramidase, and (ii) a long-term regulation by gene transcription, as documented for the first time in this study.

Furthermore, our data reveal that p38 MAPK is critically involved in the up-regulation of IL-1β-induced neutral ceramidase activity. As we (38) and others (37) have previously reported, IL-1β indeed potently activates the p38 MAPK pathway in mesangial cells. p38 MAPK has been attributed an important role in transcription of many genes (39, 40) due to its ability to phosphorylate and activate various transcription factors, including activating transcription factor-2, myocyte enhancer factor-2C, and CHOP/GADD153, which is a member of the CAAT/enhancer-binding protein family of transcription factors. Furthermore, p38 MAPK can phosphorylate and activate MAPKAPK-2, which in turn can phosphorylate transcription factors, including cAMP response element-binding protein and activating transcription factor (34), and thereby activate gene transcription.

Using mesangial cells from MAPKAPK-2 knockout mice (33), we can, however, exclude the involvement of MAPKAPK-2 in the cytokine-induced up-regulation of neutral ceramidase. The exact pathway by which p38 MAPK up-regulates the neutral ceramidase protein and activity is presently under investigation.

Our data further suggest an inverse correlation between neutral ceramidase activity and the rate of apoptosis. Whenever increased neutral ceramidase activity is observed, such as after IL-1β treatment, no DNA fragmentation can be detected (26). However, when neutral ceramidase activity is blocked by coin incubation with the p38 MAPK inhibitor SB 202190, ceramide levels (Fig. 7C) and also DNA fragmentation (data not shown) are increased. Moreover, when neutral ceramidase ac-
activity drops, such as after nitric oxide donor treatment, an increased rate of DNA fragmentation is observed (26). However, a causative role of increased ceramide levels in cell death induction is controversial and not yet settled. Some studies...
have shown that ceramide activates different caspases and thereby feeds the signal into the apoptotic pathway (41–44). Other reports have documented that ceramide can inhibit the survival signal resulting from the phosphatidylinositol 3-kinase cascade (45, 46), and it may well be that turning off a survival signal will finally direct a cell toward apoptosis. Further evidence for an involvement of ceramidases in cell survival has recently been forwarded by Strelow et al. (47). These authors showed that overexpression of the acid ceramidase in fibroblast L292 cells leads to reduced TNF-α-induced apoptosis. In contrast, Tohyama et al. (48) and Segui et al. (49) reported that fibroblasts (48) and lymphoid cells (49) from patients suffering from Farber’s disease, which results in a deficiency in acid ceramidase activity, do not show enhanced rates of apoptosis compared with healthy controls.

Another interesting observation of this study is the appearance of a double band in Northern blot analysis (Fig. 6B) as well as Western blot analysis (Fig. 4A). It is very tempting to speculate that these two bands represent different splice variants or isoforms of the neutral ceramidase. However, additional work is needed to unambiguously define the identity of both bands. In any case, the pathways controlling ceramide levels in cells exposed to stressful stimuli such as inflammatory cytokines seem to exert a stringent control on both the cera-
neutral ceramidase activity was 38.4 pmol/mg/h, and the activity in control MAPKAPK-2−/− cells was 20.1 ± 4.5 pmol/mg/h. *, p < 0.05; **, p < 0.01 (statistically significant difference compared with the corresponding unstimulated controls).

mide-generating enzymes as well as the ceramide-metabolizing enzymes, thus arguing for the relevance of ceramide and the products derived thereof for cellular functions.

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