The sphingomyelin–ceramide pathway participates in cytokine regulation of C-reactive protein and serum amyloid A, but not α-fibrinogen

Gerard LOZANSKI[1], Franck BERTHIER[2] and Irving KUSHNER[3]

Case Western Reserve University at MetroHealth Medical Center, 2500 MetroHealth Drive, Cleveland, OH 44109-1998, U.S.A.

Maximal induction of the acute-phase proteins C-reactive protein (CRP) and serum amyloid A (SAA) in the human hepatoma cell line Hep3B requires the combination of interleukin (IL)-6 and IL-1. In contrast, IL-1 inhibits fibrinogen induction by IL-6. To explore the possible participation of the sphingomyelin–ceramide pathway in the transduction of cytokine effects, the role of this pathway in expression of CRP, SAA and α-fibrinogen was investigated. The cell-permeable ceramide analogues C_2 and C_6 each greatly potentiated induction of both CRP and SAA mRNA by IL-6 + IL-1β but did not affect the responses of α-fibrinogen to IL-6 or to IL-6 + IL-1β. The combination of IL-6 + IL-1β led to increased turnover of sphingomyelin in Hep3B cells. D609, an inhibitor of ceramide production by acidic but not neutral sphingomyelinases, substantially inhibited induction of CRP and SAA by IL-6 + IL-1β. The ability of C_2 and C_6 to potentiate the effects of cytokines suggests that the sphingomyelin–ceramide pathway participates in induction of CRP and SAA by IL-6 + IL-1β under these experimental conditions, most likely by transducing the effects of IL-1β. C_2 and C_6 were unable to substitute for IL-1β in enhancing IL-6 effects on CRP and SAA, consistent with other reports indicating that the sphingomyelin–ceramide pathway is only a single component of multiple necessary converging pathways for induction of many genes. In contrast, this pathway does not appear to participate in mediating the inhibitory effects of IL-1β on fibrinogen induction by IL-6.

INTRODUCTION

Induction of acute-phase proteins in hepatocytes is primarily regulated by inflammation-associated cytokines, notably interleukin (IL)-6 and IL-1, as well as by cytokine modulators and endocrine hormones [1,2]. The role played by IL-1 in combination with IL-6 is of particular interest. In Hep3B cells, IL-1 has a marked synergistic effect on induction of the two major human acute-phase proteins, C-reactive protein (CRP) and serum amyloid A (SAA) by IL-6 [3,4]. In contrast, IL-1 inhibits induction of fibrinogen by IL-6 [5–7].

Although substantial progress has been made in defining the signal-transducing pathways that mediate induction of acute-phase proteins by IL-6 [8–11], the signal-transducing mechanisms by which IL-1 influences gene expression are much less well understood. In various model systems, binding of IL-1 to the type I IL-1 receptor appears to activate several diverging signal-transducing systems, including G-proteins, the cAMP/protein kinase A pathway and a variety of kinases and lipid-derived mediators, with ultimate activation of a number of transcription factors, including members of the nuclear factor (NF)κB, C/EBP and activator protein-1 families [12,13].

In the last few years, attention has focused on the role of the sphingomyelin signalling pathway in IL-1 signal transduction in a wide variety of cell types. Recent data indicate that ceramide, derived from degradation of sphingomyelin by activation of neutral or acidic sphingomyelinases, participates in mediating some of the pleiotropic responses to IL-1, including regulation of cell proliferation and differentiation, inflammation and apoptosis [14–17]. The current study was undertaken to explore the possibilities that ceramide might participate in induction of CRP and SAA by IL-6 + IL-1β, and in transduction of the inhibitory effect of IL-1β on fibrinogen induction by IL-6. We also attempted to determine whether acidic sphingomyelinase or one of the neutral sphingomyelinases was responsible for ceramide production in this system.

EXPERIMENTAL

Cell culture and biological reagents

The human hepatoma cell line Hep3B (American Type Culture Collection, Rockville, MD, U.S.A.) was maintained in RPMI-1640 (Bio-Whittaker, Walkersville, MD, U.S.A.) supplemented with 10% (v/v) fetal bovine serum (JRH Biosciences, Lenexa, KS, U.S.A.) in a humidified incubator containing 5% CO_2. Cells were passaged weekly by trypsinization at a 1:10 ratio. Baculovirus-expressed human recombinant IL-6 was generously provided by Dr. L. May (Valhalla, NY, U.S.A.); human recombinant IL-1β was generously provided by Genzyme (Boston, MA, U.S.A.); dexamethasone sodium salt was from American Reagent Laboratories (4 mg/ml, injection United States Pharmacopoeia; Shipley, NY, U.S.A.); the cell-permeable ceramide analogues C_2, C_6 and dihydroceramide (DHC) were purchased from BIOMOL (Plymouth Meeting, PA, U.S.A.); and compound D609 (tricyclodecan-9-yl-xanthogenate) was purchased from Sigma (St. Louis, MO, U.S.A.).

Abbreviations used: CRP, C-reactive protein; SAA, serum amyloid A; IL, interleukin; NF, nuclear factor; DHC, dihydroceramide; PC-PLC, phosphatidylcholine-specific phospholipase C; TNF, tumour necrosis factor.

[1] Present address: Summa Health System, Pathology Department, 525 E. Market St., Akron, OH 44309, U.S.A.

[2] Present address: Centre Hospitalier Lyon-Sud, Laboratoire Immunologie, 69480 Pierre Benite, France

[3] To whom correspondence should be addressed.

Evaluation of effects of ceramide analogues and the enzyme inhibitor D609

Confluent Hep3B monolayers in 35-mm-diameter six-well plates (6 days following splitting) were washed three times with prewarmed RPMI-1640, followed by addition of 1 ml of inducing medium, consisting of RPMI-1640 with IL-6 (200 units/ml), IL-1β (400 units/ml) or their combination. All experiments were carried out in the presence of 1 μM dexamethasone. The ceramide analogues C_2 and C_6 and the inactive ceramide analogue DHC were dissolved in absolute ethanol at a concentration of 5 mg/ml. Ceramides were added to a final concentration of 10 μM; the equivalent volume of absolute ethanol was added to matching control dishes. The total content of ethanol did not exceed 0.1 % (v/v).

We tested the effect of D609, a methylxanthine reported to inhibit activation of acidic but not basic sphingomyelinase by blocking phosphatidylcholine-specific phospholipase C (PC-PLC) activity [18]. Confluent Hep3B cells were washed three times with prewarmed RPMI-1640, then treated with 1 ml of RPMI-1640 medium containing 5 μg/ml D609. After 1 h, media were replaced with fresh medium containing cytokines, with or without 5 μg/ml of D609.

In all circumstances, after 24 h of incubation, media were collected and centrifuged for 5 min at 1000 rev./min. The supernatants were collected and frozen for future protein analyses. Cells were washed twice with cold PBS, and total RNA was then isolated according to the method of Chomczynski and Sacchi [19]. In preliminary experiments, no cytotoxic effects of these compounds were observed, employing Trypan Blue and the tetrazolium assay as described below.

Northern-blot analysis

RNA samples (20 μg per lane) were heat denatured for 5 min at 65 °C in a solution consisting of 50 % (v/v) deionized formamide, 2 M formaldehyde in 0.2 M Mops buffer, pH 7.0, and 10 mM EDTA, and then fractionated on 0.9 % agarose gels containing 2.2 M formaldehyde. After transfer to a MagnaGraph membrane (Micron Separations, Westboro, MA, U.S.A.) by vacuum blotting, RNA was UV cross-linked to the membrane. Dried membranes were photographed under UV translumination for the purpose of future normalization to 18 S rRNA signal intensity. Pre-hybridization (4 h) and hybridization (18 h) with CRP or SAA cDNA probes labelled with [^{32}P]dCTP by the random-primer method (10^6 c.p.m.) were performed at 42 °C in hybridization buffer consisting of 50 % (v/v) deionized formamide, 5 × SSC (1 × SSC is 0.15 M NaCl/0.015 M sodium citrate), 1 × Denhardt's solution [0.02 % (w/v) Ficoll 400/0.02 % (w/v) polyvinylpyrrolidone/0.002 % (w/v) BSA], 1 % (w/v) SDS and 10 μg/ml denatured salmon sperm DNA. Following hybridization, membranes were washed twice for 30 min with 6 × SSC/0.1 % (w/v) SDS at room temperature and twice for 15 min with 0.1 × SSC/0.1 % (w/v) SDS at 62 °C, and then exposed at −70 °C to X-Omat AR Kodak film. Membranes were stripped of probe by a 5 min exposure to 95 °C distilled water containing 1 % (w/v) SDS before hybridization with the next labelled probe. The probes employed were: SAA, pA10 (capable of detecting all SAA isotypes), courtesy of J. Sipe (Boston, MA, U.S.A.) [20]; CRP, pCRP5 kindly provided by Dr. H. R. Colten (St. Louis, MO, U.S.A.) [21]; glyceraldehyde-3-phosphate dehydrogenase, pHIcGAp3, courtesy of R. Wu (New York, NY, U.S.A.) [22]. Autoradiographic signals were measured with a UMAX scanner (Data System, Hsinchu, Taiwan) and normalized to the intensity of 18 S rRNA signals.

CRP protein measurements

CRP protein concentrations were determined using a CRP ELISA, as described previously [23]. Briefly, Immulon 2 plates were covered with 100 μl of polyclonal sheep anti-human CRP (Cappel, Durham, NC, U.S.A.) in Tris-buffered saline, pH 7.4, at a concentration of 1 μg/100 μl. Coating was allowed for 24 h at 4 °C, followed by blocking with 3 % (w/v) BSA (fat-free BSA; Calbiochem, La Jolla, CA, U.S.A.) in Tris-buffered saline, 250 μl/well for 4 h at 37 °C. As a second antibody, purified goat anti-(human CRP) IgG (Atlantic Antibodies, Stillwater, MN, U.S.A.) was used. Following biotinylation, the optimal concentration of the second antibody was established using serial dilutions of a highly purified preparation of human CRP as a standard. The assay was linear in the range of 0.5–12 ng/ml of antigen.

Assay of sphingomyelin turnover

To determine effects of cytokines on cellular sphingomyelin turnover, we used a modification of the method of Jayadev et al. [24]. Hep3B cells were grown to confluence (6 days) in RPMI-1640 medium containing 10 % (v/v) fetal bovine serum, penicillin G (100 units/ml), streptomycin (100 μg/ml) and Amphotericin B (0.25 μg/ml) in 5 % CO_2 at 37 °C. The cells were then washed three times with RPMI-1640 and incubated with [^{3}H]choline chloride (1 μCi/ml; specific activity 81.0 Ci/mmol; Du Pont NEN, Wilmington, DE, U.S.A.) in serum-free RPMI-1640 medium for 48 h. We then added IL-6 + IL-1β to appropriate dishes. Incubation was stopped either immediately (zero time) or after 2 h by removing the incubation medium and adding 0.5 ml of a cold lysing buffer composed of 0.1 M Tris/HCl, pH 7.4, and 0.1 % (v/v) Triton X-100. The cells were then detached from the dishes and placed in 1.7 ml tubes, vortexed and kept at 4 °C for 5 min, after which lipids were immediately extracted by addition of 0.9 ml of chloroform/methanol (2:1; v/v). These organic-phase samples were dried under air flow and redissolved in 0.1 ml of lysing buffer.

An enzymic method was employed to determine the amount of labelled sphingomyelin remaining in the lipid extract. We added 100 μl of neutral buffer (0.1 M Tris/HCl, pH 7.4, and 5 mM $MgCl_2$) and 0.1 unit of neutral sphingomyelinase (S-8633; Sigma) to the 100 μl of redissolved chloroform/methanol extract and incubated the mixture for 30 min at 37 °C. A second lipid extraction with 0.75 ml of chloroform/methanol was then carried out, and the [^{3}H]phosphorylcholine content of the aqueous phase was determined by adding 100 μl of the upper liquid layer to 10 ml of scintillation liquid (ScintiVerse II; Fisher Scientific, Pittsburgh, PA, U.S.A.) and counting for radioactivity in a scintillation counter. For each sample, background radioactivity was subtracted (lipids extracted from cells without addition of neutral sphingomyelinase). The positive control for the assay was 0.1 unit of a purified neutral sphingomyelinase added to 1 ml of [*N*-methyl-^{14}C]sphingomyelin (bovine; 56 mCi/mmol; Amersham, Arlington Hts., IL, U.S.A.). Independent experiments were performed on three different days, in duplicate on two days and in triplicate once.

Viability and metabolic integrity

Possible toxic effects of ceramide analogues and D609 were evaluated following 24 h exposure by measuring cell viability by the Trypan Blue exclusion test and by assessment of metabolic integrity by the tetrazolium assay [25]. No toxic effects were observed in the doses employed.

RESULTS

The effects of the ceramide analogues C_2, C_6 and DHC on cytokine-induced changes in expression of CRP, SAA and α-fibrinogen mRNAs are shown in Figure 1. In this series of experiments, induction of both CRP and SAA required the combination of IL-6 and IL-1β, neither cytokine alone producing detectable induction. Addition of the active ceramide analogues C_2 or C_6 to Hep3B cells treated with IL-6 + IL-1β potentiated CRP induction by 73 and 89 % respectively and potentiated SAA induction by 93 and 116 % respectively, whereas addition of the inactive ceramide analogue DHC had no effect on either CRP or SAA mRNA levels (Table 1). Secretion of CRP into medium paralleled mRNA levels in these studies (results not shown). C_2 and C_6 were unable to substitute for IL-1β in enhancing IL-6 effects on CRP and SAA induction (Figure 1). In contrast, α-fibrinogen was induced by IL-6 alone, whereas IL-1β decreased basal expression of α-fibrinogen mRNA and partially inhibited IL-6 induction of this gene (Figure 1 and Table 1). The effects of cytokines on α-fibrinogen expression were not affected by any of the three ceramide analogues (Table 1).

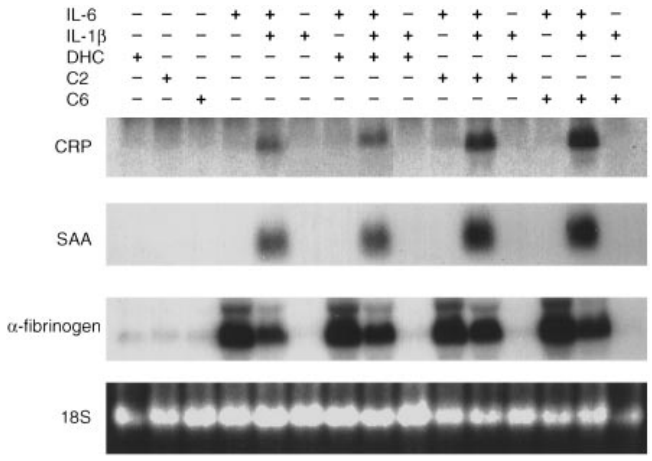

Figure 1 Effects of ceramide analogues on CRP, SAA and α-fibrinogen mRNA accumulation

Representative Northern blot showing effects of the active short-chain ceramide analogues C_2 and C_6 and the inactive analogue DHC on CRP, SAA and α-fibrinogen mRNA accumulation in Hep3B cells incubated with cytokines. Confluent monolayers of Hep3B cells were incubated with 200 units/ml IL-6, 400 units/ml IL-1β or their combination, with or without 10 μM C_2, C_6 or DHC. Following 24 h of incubation, total RNA was isolated, and the abundance of CRP, SAA and α-fibrinogen mRNA was estimated using specific cDNA probes. The intensity of the 18 S signal was used to adjust for variable lane loading.

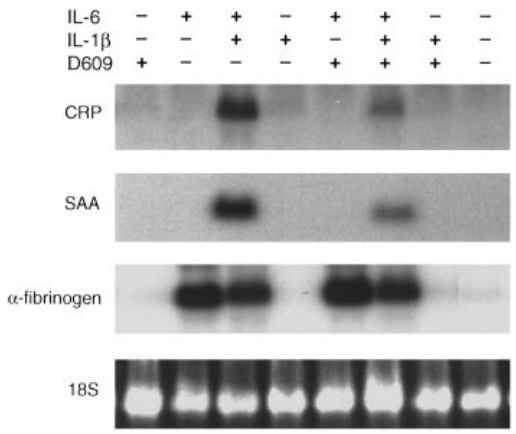

Figure 2 Representative Northern blot showing effects of D609 on cytokine induction of CRP, SAA and α-fibrinogen

Confluent monolayers of Hep3B cells were treated with 5 μg/ml D609 1 h before and during 24 h of induction with IL-6 or IL-6 + IL-1β. Total RNA was then extracted, and the abundance of CRP, SAA and α-fibrinogen mRNA was estimated by the Northern-hybridization technique using specific cDNA probes. The intensity of the 18 S signal was used to adjust for variable lane loading.

The effects of the PC-PLC inhibitor D609, an inhibitor of acidic sphingomyelinase activation, on cytokine-induced expression of CRP, SAA and α-fibrinogen are shown in Figure 2 and Table 2. Addition of D609 to cells treated with IL-6 + IL-1β resulted in an 86 % decrease in CRP mRNA abundance and an 88 % decrease in SAA mRNA abundance in three experiments. Secretion of detectable CRP into medium in response to IL-6 + IL-1β was almost completely abolished by D609 treatment (results not shown). Even in doses of 50 μg/ml, C_2 and C_6 were not able to overcome the inhibitory effects of D609 (results not shown). D609 had no effect on either α-fibrinogen induction by IL-6 or on the inhibitory effect of IL-1β on this induction.

The effect of IL-6 + IL-1β on sphingomyelin turnover in Hep3B cells was determined. Following radiolabelling of cellular sphingomyelin by 48 h of exposure to [^{3}H]choline chloride, incubation

Table 1 Influence of short-chain ceramide analogues on acute-phase protein mRNA accumulation

Confluent monolayers of Hep3B cells were incubated for 24 h with 200 units/ml IL-6, or this concentration of IL-6 + 400 units/ml IL-1β, with or without 10 μM C_2, C_6 or DHC. Total RNA was harvested and subjected to Northern analysis. Results are expressed as the percentage of RNA/18 S seen with IL-6 + IL-1β for CRP and SAA and that seen with IL-6 alone for α-fibrinogen. Results are the mean values of three experiments. –, none detected.

	mRNA accumulation (mean % ± S.E.M.)							
Ceramide analogue...	None		C_2		C_6		DHC	
IL-6...	+	+	+	+	+	+	+	+
IL-1β...	0	+	0	+	0	+	0	+
CRP	–	100	–	173 ± 11	–	189 ± 17	–	97 ± 13
SAA	–	100	–	193 ± 9	–	216 ± 12	–	107 ± 16
α-Fibrinogen	100	62 ± 4.7	97 ± 5.0	57 ± 10.5	104 ± 8.0	69 ± 7.0	107 ± 3.5	56 ± 9.5

Table 2 Effects of D609 on acute-phase protein mRNA abundance

Confluent monolayers of Hep3B cells were treated with 5 μg/ml D609 1 h before and during 24 h of incubation with IL-6 or IL-6 + IL-1β. Normalized densitometric units (ratio of specific signal to 18 S) were transformed into percentages, where values from Hep3B cells treated with IL-6 + IL-1β for CRP amd SAA or with IL-6 alone for α-fibrinogen were 100%. Results are the mean values of three experiments. –, none detected.

	mRNA abundance (mean% ± S.E.M.)							
D609...	0	+	0	0	0	+	+	+
IL-6...	0	0	+	+	0	+	+	0
IL-1β...	0	0	0	+	+	0	+	+
CRP	–	–	–	100	–	–	14 ± 11.0	–
SAA	–	–	–	100	–	–	12 ± 17.8	–
α-Fibrinogen	4 ± 1.2	3.75 ± 0.7	100	57.7 ± 6.0	0	102 ± 9.0	64 ± 3.7	0

of Hep3B cells with IL-6 + IL-1β for 2 h resulted in a decrease of 22% (±6.5 S.E.M.; $P < 0.05$) in labelled sphingomyelin. No significant change in labelled sphingomyelin was observed in cells maintained for the same 2 h period in the absence of cytokines.

DISCUSSION

A growing body of evidence indicates that some, but not all, of the effects of IL-1 are transduced by activation of sphingomyelinases, with consequent production of ceramide [13–17,26]. The current study was undertaken to evaluate the possibilities that a sphingomyelinase–ceramide pathway might participate in mediation of the synergistic effects of IL-1 on induction of CRP and SAA by IL-6, on the inhibitory effects of IL-1 on fibrinogen induction, or both. Our major conclusions are that the sphingomyelin pathway participates in mediation of the effects of IL-1β on induction of CRP and SAA, but not in mediating the inhibitory effects of IL-1β on fibrinogen expression.

The finding that addition of the cell-permeable active ceramide analogues C_2 and C_6 markedly potentiated induction of CRP and SAA mRNA by IL-6 + IL-1β, with comparable increases in secreted CRP production, whereas the inactive analogue DHC had no effect, indicates that ceramide participates in transducing the inducing effects of these cytokines on CRP and SAA. Since detectable induction of CRP and SAA in Hep3B cells, in the current set of experiments, required the combination of IL-6 and IL-1β, it was not possible to evaluate the effects of the ceramide analogues on changes induced by the individual cytokines. However, we attribute the potentiating effect of C_2 and of C_6 to the IL-1β signal-transducing pathway, since sphingomyelinase activation by IL-1 is well recognized in a wide variety of cell types, whereas no such activity has been reported for IL-6. For the same reasons, we attribute the increased sphingomyelin turnover induced by this combination of cytokines to IL-1β and not to IL-6. Increased sphingomyelin turnover induced by IL-1 has previously been demonstrated in rat hepatocytes [27].

Neither C_2 nor C_6 could mimic the effects of IL-1β in synergizing induction of CRP and SAA by IL-6, but both potentiated this effect. Although this finding is not in accord with many reports in which cell-permeable ceramide analogues were able to mimic cytokine effects (e.g. [27–29]), there are ample precedents in which ceramide analogues, ineffective alone, are found to enhance cytokine effects. Thus an enhancing effect of C_2 on IL-1-induced prostaglandin E2 production in fibroblasts has been shown, whereas C_2 alone had no effect [30]. Similarly, ceramide analogues enhanced IL-1-induced E-selectin expression in endothelial cells, but had no effect in the absence of IL-1 [31], and a ceramide analogue was found to have only a minimal enhancing effect on NFκB activation in HL-60 cells, but a major potentiating effect in the presence of tumour necrosis factor (TNF)-α [32]. C_2 alone was unable to activate NFκB, but did enhance NFκB activation in response to TNF-α [33]. Finally, Higuchi et al. [34] found that acidic sphingomyelinase-generated ceramide was necessary, but not sufficient, for TNF-α-induced apoptosis and NFκB activation, suggesting that other factors activated by TNF-α synergize with ceramide. Our finding suggests that the ceramide pathway alone is not sufficient to transduce the effects of IL-1β on the CRP and SAA genes and that other IL-1β-activated converging signal-transducing pathways are required for these effects. The importance of converging pathways in ceramide-induced effects is increasingly becoming apparent; although ceramide appears to be involved in apoptotic signalling for Fas, it is clear that other signals are necessary for Fas-mediated apoptosis [35].

Ceramide can be derived from the effects of several different sphingomyelinases on sphingomyelin. Two different neutral and one acidic sphingomyelinase activities have been found, and a gene coding for acidic sphingomyelinase has been identified [36]. Acidic sphingomyelinase resides in caveoli, endosomes and lysosomes [37,38]. It appears to be activated by a pathway in which cytokine binding to receptor leads to activation of PC-PLC, with generation of 1,2-diacylglycerol, which in turn activates acidic sphingomyelinase, with consequent ceramide production [39]. D609 is a methylxanthine which is reported to inhibit PC-PLC specifically and thus block production of 1,2-diacylglycerol and, hence, of acidic, but not neutral, sphingomyelinase [18]. Our finding that induction of CRP and SAA by IL-6 + IL-1β is substantially inhibited by D609 is consistent with the possibility that the ceramide that mediates induction of these two genes is formed as a result of activation of acidic sphingomyelinase, rather than one of the neutral sphingomyelinases. However, this can only be a tentative conclusion, since D609 is also capable of inhibiting other enzymes, such as phospholipase D, phospholipase C and nitric oxide synthase [40,41], which could conceivably participate in mediating induction of CRP and SAA by cytokines.

In contrast with their effects on induction of CRP and SAA, C_2 and C_6 had no effect on either induction of α-fibrinogen by IL-6 or inhibition of this induction by IL-1β. Failure of C_2-ceramide to influence induction of β-fibrinogen by IL-6 in rat hepatocytes cells has previously been reported [27]. These observations suggest that ceramide does not participate in the signal-transducing pathways by which these cytokine effects (particularly IL-1β effects) on α-fibrinogen are mediated and that other signal-transducing pathways mediate this process. These findings under-

score the divergent nature of the signal-transducing pathways resulting from binding of IL-1β to its cognate receptor. Similarly, the TNF receptor is known to utilize both ceramide-dependent and -independent pathways [42–44].

It should be emphasized that the observation that ceramide participates in induction of CRP and SAA by IL-6+IL-1β but that ceramide analogues cannot substitute for cytokines indicates the importance of converging signal-transducing mechanisms in induction of these acute-phase proteins, whereas the observation that ceramide does not participate in α-fibrinogen changes produced by the same cytokines underscores the divergent nature of the signal-transduction pathways resulting from ligation of a cytokine to its cognate receptor.

Relatively little is known about the immediate downstream targets for ceramide signalling, which are felt to include, among others, a variety of kinases and phosphatases [30,37,45]. It was previously observed that okadaic acid, a potent inhibitor of phosphoserine phosphothreonine protein phosphatases 1 and 2A, is able to inhibit strongly induction of CRP by IL-6+IL-1β in Hep3B cells [46]. Our current findings raise the possibility that this effect of okadaic acid might have resulted from inhibition of a ceramide-activated phosphatase, since okadaic acid has been found to abrogate certain ceramide-mediated effects [47], although other explanations are clearly possible. Since SAA induction by IL-1 has clearly been shown to involve NFκB activation [48], a recognized effect of acid sphingomyelinase activation in some model systems [18], the role of ceramide in SAA induction may be to mediate NFκB activation. On the other hand, an explanation for the role of ceramide in CRP induction must be more speculative, since a role for NFκB in CRP induction has not been demonstrated.

It should be noted that CRP induction in Hep3B cells has been found, in several laboratories, to require the combination of IL-6+IL-1 [49,50], the effect of IL-6 alone being minimal at best [4]. In contrast, IL-6 alone can markedly induce CRP in NPLC/PRF/5 cells [49] and in human primary hepatocyte cultures [51]. In none of these model systems can we be confident that the findings truly reflect *in vivo* events, since there are sources of error intrinsic to each [52]. Nonetheless, studies in immortalized cell lines such as Hep3B, even if not precisely replicating the usual processes operative *in vivo*, can provide insight into the possible mechanisms which can regulate gene expression in normal hepatocytes.

This research was supported by National Institutes of Health Grant AG-02467. We gratefully acknowledge the technical and editorial assistance of Debra Rzewnicki. We appreciate the editorial comments of Martin Snider and Mark Kester.

REFERENCES

1 Kushner, I. and Rzewnicki, D. (1997) in Fever: Basic Mechanisms and Management, (Mackowiak, P. A., ed.), pp. 165–176, Lippincott-Raven, Philadelphia
2 Kushner, I. and Rzewnicki, D. (1994) Bailliere's Clin. Rheumatol. **8**, 513–530
3 Jiang, S., Lozanski, G., Samols, D. and Kushner, I. (1995) J. Immunol. **154**, 825–831
4 Zhang, D., Jiang, S., Rzewnicki, D., Samols, D. and Kushner, I. (1995) Biochem. J. **310**, 143–148
5 Gauldie, J., Richards, C., Harnish, D., Lansdorp, P. and Baumann, H. (1987) Proc. Natl. Acad. Sci. U.S.A. **84**, 7251–7255
6 Andus, T., Geiger, T., Hirano, T., Kishimoto, T. and Heinrich, P. C. (1988) Eur. J. Immunol. **18**, 739–746
7 Mackiewicz, A., Speroff, T., Ganapathi, M. K. and Kushner, I. (1991) J. Immunol. **146**, 3032–3037
8 Zhang, D., Sun, M., Samols, D. and Kushner, I. (1996) J. Biol. Chem. **271**, 9503–9509
9 Ihle, J. N. (1995) Nature (London) **377**, 591–594
10 Kishimoto, T., Akira, S., Narazaki, M. and Taga, T. (1995) Blood **86**, 1243–1254
11 Ramji, D. P., Cortese, R. and Ciliberto, G. (1995) in Acute-phase Proteins. Molecular Biology, Biochemistry, and Clinical Applications, (Mackiewicz, A., Kushner, I. and Baumann, H., eds.), pp. 365–396, CRC Press, Boca Raton
12 Bankers-Fulbright, J. L., Kalli, K. R. and McKean, D. J. (1996) Life Sci. **59**, 61–83
13 Dinarello, C. A. (1994) FASEB J. **8**, 1314–1325
14 Spiegel, S., Foster, D. and Kolesnick, R. (1996) Curr. Opin. Cell Biol. **8**, 159–167
15 Serhan, C. N., Haeggström, J. Z. and Leslie, C. C. (1996) FASEB J. **10**, 1147–1158
16 Ballou, L. R., Laulederkind, S. J. F., Rosloniec, E. F. and Raghow, R. (1996) Biochim. Biophys. Acta **1301**, 273–287
17 Verheij, M., Bose, R., Lin, X. H., Yao, B., Jarvis, W. D., Grant, S., Birrer, M. J., Szabo, E., Zon, L. I., Kyriakis, J. M. et al. (1996) Nature (London) **380**, 75–79
18 Wiegmann, K., Schütze, S., Machleidt, T., Witte, D. and Krönke, M. (1994) Cell **78**, 1005–1015
19 Chomczynski, P. and Sacchi, N. (1987) Anal. Biochem. **162**, 156–159
20 Sipe, J. D., Colten, H. R., Goldberger, G., Edge, M. D., Tack, B. F., Cohen, A. S. and Whitehead, A. S. (1985) Biochemistry **24**, 2931–2936
21 Tucci, A., Goldberger, G., Whitehead, A. S., Kay, R. M., Woods, D. E. and Colten, H. R. (1983) J. Immunol. **131**, 2416–2419
22 Tso, J. Y., Sun, X., Kao, T., Reece, K. S. and Wu, R. (1985) Nucleic Acids Res. **13**, 2485–2502
23 Drenth, J. P. H., Van der Meer, J. W. M. and Kushner, I. (1996) J. Immunol. **157**, 400–404
24 Jayadev, S., Linardic, C. A. and Hannun, Y. A. (1994) J. Biol. Chem. **269**, 5757–5763
25 Price, P. and McMillan, T. J. (1990) Cancer Res. **50**, 1392–1396
26 Kuno, K. and Matsushima, K. (1994) J. Leuk. Biol. **56**, 542–547
27 Chen, J. Q., Nikolova-Karakashian, M., Merrill, A. H. and Morgan, E. T. (1995) J. Biol. Chem. **270**, 25233–25238
28 Fiebich, B. L., Lieb, K., Berger, M. and Bauer, J. (1995) J. Neuroimm. **63**, 207–211
29 Sjöholm, Å. (1995) FEBS Lett. **367**, 283–286
30 Ballou, L. R., Chao, C. P., Holness, M. A., Barker, S. C. and Raghow, R. (1992) J. Biol. Chem. **267**, 20044–20050
31 Masamune, A., Igarashi, Y. and Hakomori, S. (1996) J. Biol. Chem. **271**, 9368–9375
32 Boland, M. P., Foster, S. J. and O'Neill, L. A. J. (1996) Biochem. Soc. Trans. **24**, 1S
33 Dbaibo, G. S., Obeid, L. M. and Hannun, Y. A. (1993) J. Biol. Chem. **268**, 17762–17766
34 Higuchi, M., Singh, S., Jaffrezou, J. and Aggarwal, B. B. (1996) J. Immunol. **156**, 297–304
35 Skowronski, E. W., Kolesnick, R. N. and Green, D. R. (1996) Cell Death Diff. **3**, 171–176
36 Schuchman, E. H., Suchi, M., Takahashi, T., Sandhoff, K. and Desnick, R. J. (1991) J. Biol. Chem. **266**, 8531–8539
37 Testi, R. (1996) Trends Biol. Sci. **21**, 468–471
38 Liu, P. and Anderson, R. G. (1995) J. Biol. Chem. **270**, 27179–27185
39 Schütze, S., Machleidt, T. and Krönke, M. (1994) J. Leuk. Biol. **56**, 533–541
40 Kiss, Z. and Tomono, M. (1995) Biochim. Biophys. Acta **1259**, 105–108
41 Tschaikowsky, K., Meisner, M., Schonhuber, F. and Rugheimer, E. (1994) Br. J. Pharmacol. **113**, 664–668
42 Modur, V., Zimmerman, G. A., Prescott, S. M. and Mcintyre, T. M. (1996) J. Biol. Chem. **271**, 13094–13102
43 Huwiler, A., Brunner, J., Hummel, R., Vervoordeldonk, M., Stabel, S., Vandenbosch, H. and Pfeilschifter, J. (1996) Proc. Natl. Acad. Sci. U.S.A. **93**, 6959–6963
44 Bourteele, C., Röpke, C., Ayoub, M., Pfizenmaier, K. and Müller, G. (1996) Eur. Cytokine Net. **7**, 68
45 Pushkareve, M., Obeid, L. M. and Hannun, Y. A. (1995) Immunol. Today **16**, 294–297
46 Ganapathi, M. K. (1992) Biochemistry **284**, 645–648
47 Hannun, Y. A., Obeid, L. M. and Dbaibo, G. S. (1996) in Handbook of Lipid Research, (Bell, R. M., Exton, J. H. and Prescott, S. M., eds.), pp. 177–204, Plenum Press, New York
48 Betts, J. C., Cheshire, J. K., Akira, S., Kishimoto, T. and Woo, P. (1993) J. Biol. Chem. **268**, 25624–25631
49 Ganapathi, M. K., May, L. T., Schultz, D., Brabenec, A., Weinstein, J., Sehgal, P. B. and Kushner, I. (1988) Biochem. Biophys. Res. Commun. **157**, 271–277
50 Ganter, U., Arcone, R., Toniatti, C., Morrone, G. and Ciliberto, G. (1990) EMBO J. **8**, 3773–3779
51 Castell, J. V., Gomez-Lechon, M. J., David, M., Fabra, R., Trullenque, R. and Heinrich, P. C. (1990) Hepatology **12**, 1179–1186
52 Kushner, I. (1989) Ann. N. Y. Acad. Sci. **557**, 240–242

Received 9 April 1997/19 June 1997; accepted 18 July 1997