Sphingosine Synergistically Stimulates Tumor Necrosis Factor α-induced Prostaglandin E_2 Production in Human Fibroblasts

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Summary

Sphingosine is a biologically active derivative of sphingomyelin. It affects diverse cellular functions and its mechanism(s) of action is poorly defined. Tumor necrosis factor α (TNFα) has recently been shown to rapidly induce sphingomyelin turnover, implicating this metabolic pathway in TNFα signal transduction. Because TNFα is known to induce prostaglandin E_2 (PGE_2) production in human fibroblasts, we tested the effect of sphingosine on TNFα-induced PGE_2 production. We found that sphingosine enhanced TNFα-induced PGE_2 production by as much as 18-fold over TNFα alone. Sphingosine appeared to stimulate TNFα-induced PGE_2 production independent of TNFα-mediated interleukin 1 (IL-1) production, because anti-IL-1 antibodies and IL-1 receptor antagonist protein (IRAP) did not inhibit TNFα-induced PGE_2 production or the stimulatory effect of sphingosine. TNFα stimulated PGE_2 production to the same degree in normal and protein kinase C (PKC) downregulated cells in the presence and absence of sphingosine, indicating that neither TNFα nor sphingosine require active PKC to elicit their respective effects. The sphingosine analogues stearylamine and stearoyl-D-sphingosine had little or no effect on TNFα-mediated PGE_2 production, supporting a specific role for sphingosine in the activation process. Short-term (1 min) exposure of cells to sphingosine dramatically increased TNFα-induced PGE_2 production. A potential mechanism by which sphingosine could increase TNFα-induced PGE_2 production involves enhancement of phospholipase A_2 (PLA_2) and/or cyclooxygenase (Cox) activity, the rate-limiting enzymes in PGE_2 production. We found that both TNFα and sphingosine alone enhanced these enzymatic activities, and that sphingosine additively increased the effect of TNFα on phospholipase A_2 activity. It appears that sphingosine affects TNFα-induced PGE_2 production via a mechanism that is independent of PKC involvement, and that sphingosine may function as an endogenous second messenger capable of modulating the responsiveness of the cell to external stimuli.

Sphingosine, a metabolite of sphingomyelin turnover (1), can elicit a variety of cellular responses including inhibition of growth factor action (2, 3), modulation of receptor function (4–6), inhibition of platelet and neutrophil function (7, 8) and calmodulin dependent enzymes (9), antagonism of phorbol ester induced responses (10–12), and promotion of antitumor activity (1, 13). Many of the affects of sphingosine appear to be a direct consequence of the inhibition of protein kinase C (PKC)[1] activity (7, 8, 10, 11), a key regulatory enzyme involved in signal transduction in a variety of physiological processes (14, 15). Although the full range of biochemical targets for sphingosine has yet to be identified, several PKC-independent mechanisms for sphingosine action have been recognized (16–18). The diverse effects of sphingosine suggest that it may act as an endogenous modulator of cell function, a second messenger molecule, generated as a result of agonist-stimulated sphingolipid turnover (1). However, the mechanism(s) of sphingosine action and those signal transduction pathways that it modulates are not well defined.

TNFα is a pluripotent cytokine secreted by macrophages in response to a variety of inflammatory agents (19). It has a broad range of in vivo activities as demonstrated by its ability to affect the growth, differentiation, and function of virtually every cell type investigated (20, 21). Cellular responses to TNFα are triggered by interaction of TNFα with high-affinity cell surface receptors (22, 23). Although sensitivity to TNFα can be modulated by regulation of receptor expres-

[1] *Abbreviations used in this paper:* Cox, cyclooxygenase; IRAP, IL-1 receptor antagonist protein; PGE_2, prostaglandin E_2; PKC, protein kinase C; PLA_2, phospholipase A_2.

sion, TNFα responsiveness appears to be largely determined at the post-receptor level. Several lines of evidence suggest the participation of protein kinases in TNFα signal transduction (24, 25), and other findings support protein kinase-independent mechanisms (26, 27). Recently, TNFα has been shown to stimulate sphingomyelin metabolism, suggesting that this pathway may be an important signaling mechanism mediating some of the many actions of TNFα (28).

IL-1 and TNFα are known to elicit similar responses in a variety of cell types, including stimulation of prostaglandin E_2 (PGE_2) production (29, 30). Both cytokines are elaborated by mononuclear cells in response to the same stimuli. They have similar functional profiles, molecular weights, and ionic characteristics (31–34), and further, TNFα can itself induce the production of IL-1 (35). Thus, specific responses of fibroblasts to these cytokines are sometimes difficult to differentiate. We show here that sphingosine stimulates TNFα-mediated PGE_2 production via a PKC-independent pathway, at least in part, by stimulating phospholipase A_2 (PLA_2) activity. TNFα-mediated IL-1 production does not appear to be involved in the process. Our observation that very short exposure of cells to sphingosine is sufficient to maximally stimulate TNFα-mediated PGE_2 production, together with the previous observation that TNF-α rapidly induces the metabolism of sphingomyelin, is consistent with the concept that sphingolipid metabolites may play an important role as endogenous second messengers in regulating the ability of cells to respond to TNFα and, most likely, to other cytokines.

Materials and Methods

Cytokine Preparations and Antibodies. Sphingosine from bovine brain sphingomyelin was purchased from Sigma Chemical Co., (St. Louis, MO), and recombinant TNFα was from R & D Systems (Minneapolis, MN). mAbs to IL-1α and IL-1β were obtained from R & D Systems, and IRAP (36) was the generous gift of Dr. Daniel E. Tracey of The Upjohn Company (Kalamazoo, MI). PMA, stearoyl-D-sphingosine, and stearylamine were all obtained from Sigma Chemical Co. [^{3}H]PGE_2 was purchased from New England Nuclear (Boston, MA). PGE_2 antiserums were obtained from Advanced Magnetics, Inc. (Cambridge, MA).

Fibroblasts Cell Lines and Culture. Six different human foreskin fibroblast lines were employed in these studies. Human foreskin fibroblasts were obtained and cultured as previously described (18). Where indicated cells were treated with growth factors, anti-IL-1α and anti-IL-1β mAbs (10 and 100 μg/ml, respectively), IRAP (1 ng/ml), PMA (100 nM final in DMSO), or aliphatic amines (10 μM), along with the appropriate vehicle controls. Sphingosine was always added as a complex with BSA, which was prepared by incubating equimolar amounts of BSA and sphingosine in Tris buffer (pH 7.4) for 1 h at 37°C. Incubations typically lasted 24 h after which time the media were collected and assayed for PGE_2 by RIA. The effects of sphingosine and TNFα on cell viability were assessed by trypan blue exclusion. At 10 μM sphingosine and 1 ng/ml TNFα, neither cell morphology nor viability was affected during the course of the experiments described.

PGE_2 RIA. This assay is based upon the competition of cold (sample PGE_2) with labeled PGE_2 for anti-PGE_2-antibody binding sites as previously described (18). The culture media were harvested from cells treated as indicated, and a 10-μl aliquot was added to RIA assay buffer (0.1 mM phosphate buffer, pH 7.4, containing 0.9% sodium chloride, 0.1% sodium azide, and 0.1% gelatin). The sample was then mixed with the appropriate amount of labeled tracer and reconstituted antiserum. The mixture was allowed to equilibrate at room temperature for 1 h and then incubated overnight at 4°C. Assay tubes were then placed in an ice bath, and 1 ml of a cold charcoal-dextran suspension was added. After a 15-min incubation, the tubes were centrifuged at 2,200 *g* for 10 min at 4°C; the supernatants were decanted into scintillation vials and counted using liquid scintillation. Percent binding was compared against a standard curve, and the amount of PGE_2 in the sample was calculated.

PKC Downregulation. Confluent fibroblast cultures were pretreated with 1 μM PMA for 24 h at 37°C. After this treatment, the cells were washed three times with Eagle's minimal essential medium (EMEM), and 0.5 ml fresh EMEM containing 1% FCS was added. The cells were then treated as indicated and PGE_2 was measured as described above.

PLA_2 Assay. The assay used to measure PLA_2 activity in cell extracts is a modification of a procedure previously described by Ballou, et al. (37–39). Briefly, confluent fibroblast monolayers from six-well plates were washed several times with 2 ml of 100 mM Tris, pH 7.0. The cells were scraped from the plate in 120 μl of the same buffer, sonicated for 15 s, and centrifuged at 15,000 *g* for 15 min. The pellet was discarded and the supernatant fraction kept on ice until assayed for PLA_2 activity. Each assay contained 40 μl of the supernatant fraction (~50 μg of protein), 1-palmitoyl-2-[1-^{14}C]arachidonylphosphatidylcholine (50,000 cpm in 5 μl DMSO), and 1 mM $CaCl_2$. The assay was initiated by adding 10 μl of a reaction mixture such that the final concentration of each component was as described above. Tubes were mixed by vortex and incubated in a 37°C water bath for 30 min. The reaction was stopped by the addition of 50 μl of ethanol containing 2% acetic acid. Each tube was mixed by vortex and 50 μl was applied to a silica gel G TLC plate and developed in ethyl acetate/acetic acid (99:1). As indicated for certain experiments, cells were pretreated with TNFα and/or sphingosine for 18 h before preparation of the extract.

Cox Assay. The procedure for obtaining the cell extract was the same as for the PLA_2 assay. Each Cox assay contained 40 μl of the cell extract and 1 μM epinephrine/1 mM phenol in 100 mM Tris (pH 8.5). The reaction was initiated by adding 10 μl 1 mM arachidonic acid in ethanol to each tube. The reaction was allowed to proceed for 15 min at 37°C and stopped by the addition of 10 μl 25 mM $FeCl_2$-$4H_2O$. Then, the samples were stored at −20°C until PGE_2 was measured by RIA as described above.

Statistical Analysis. Student's *t* test was used for all statistical analyses.

Results

Sphingosine Potentiates TNFα-induced PGE_2 Production in Human Fibroblasts. In a series of experiments we evaluated the effect of TNFα and sphingosine on PGE_2 production in human fibroblasts. In six cell lines tested, we consistently observed a dramatic increase in the amount of PGE_2 produced in response to TNFα if sphingosine was also present. Fig. 1 shows that at 10 μM sphingosine, approximately 18-fold more PGE_2 was produced in response to 1 ng/ml TNFα than in the absence of sphingosine. Sphingosine alone had comparatively little effect on PGE_2 production at any con-

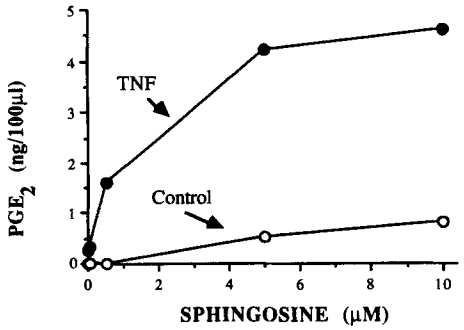

Figure 1. Sphingosine potentiates TNFα-induced PGE_2 production. Confluent human foreskin fibroblasts were treated with TNFα (1 ng/ml) and the indicated concentrations of sphingosine. PGE_2 was measured by RIA as described in Materials and Methods. The results are expressed as ng of PGE_2 produced per 100 μl. Similar results were obtained in two additional experiments. Standard deviations between duplicate assays for each experimental treatment were less than 5%.

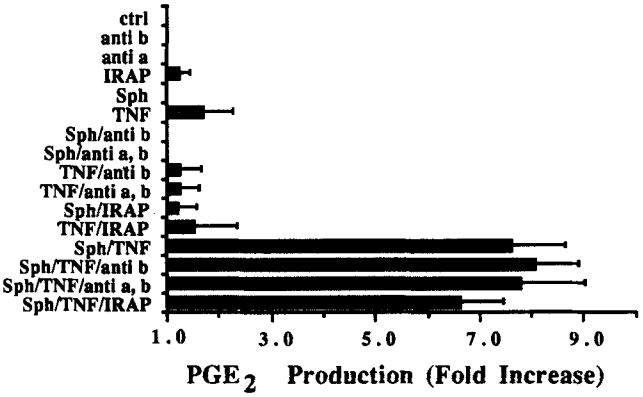

Figure 2. IRAP and mAbs against IL-1 do not inhibit TNFα-induced PGE_2 production. Confluent fibroblast cultures (24-well) were treated as indicated. After 24 h PGE_2 was measured by RIA as described in Materials and Methods. Untreated control PGE_2 levels were normalized to 1.0, and the fold stimulation by each treatment was calculated. Each value is the mean of three additional experiments from two identically treated wells ± SD. The concentration of each addition was: anti-IL-1β, 100 μg/ml; anti-IL-1α, 10 μg/ml, IRAP, 1 ng/ml; sphingosine, 10 μM; TNFα, 1 ng/ml.

centration tested. TNFα-mediated PGE_2 production increased as a function of sphingosine concentration. However, concentrations of sphingosine over 25 μM were cytotoxic, as previously reported (18). Although we observed some degree of quantitative variability with respect to PGE_2 production among the cell lines examined, the combination of TNFα and sphingosine invariably resulted in a synergistic increase of PGE_2 production. In a series of six separate experiments each using a different fibroblast cell line, we observed a sphingosine-mediated increase in TNFα-induced PGE_2 production ranging from 3.5- to 17.7-fold over TNFα levels alone. The lowest mean increase (3.5-fold) induced by TNFα and sphingosine over TNFα controls was statistically significant (Student's t test, $p < 0.005$).

IRAP and Anti-IL-1 Antibodies Do Not Inhibit TNFα/Sphingosine Synergy. TNFα has been shown to induce IL-1 synthesis by certain target cells (35). Therefore, to rule out potential effects of IL-1 on TNFα-mediated PGE_2 production, we tested the effects of sphingosine and TNFα in the presence of neutralizing IL-1 (α and β) mAbs and IRAP. We added IRAP (1 ng/ml), which blocks the action of IL-1 by binding to IL-1 receptors with about the same affinity as IL-1 (36), anti-IL-1α (10 μg/ml), and anti-IL-1β (100 μg/ml), an amount that totally blocked the effect of exogenously added IL-1 (1 ng/ml) on PGE_2 production (data not shown). Fig. 2 shows that when IRAP or anti-IL-1 antibodies are added to the incubation medium, TNFα-induced PGE_2 production remains stimulated by sphingosine. Control assays containing combinations of anti-IL-1, IRAP, sphingosine, and each alone, resulted in no effect on basal PGE_2 production. Thus, sphingosine and TNFα appear to increase PGE_2 production in human fibroblasts independent of IL-1 effects, although we cannot rule out the potential effects of intracellular IL-1.

The Effect of Sphingosine on PMA and TNFα-Mediated PGE_2 Production After PKC Downregulation. Many effects of sphingosine (7, 8, 10, 11) and TNFα (24, 25) appear to be a direct consequence of PKC inhibition or activation, respectively. PMA is a potent activator of PKC (15); however, prolonged exposure to PMA downregulates PKC activity in human cells (40, 41). Therefore, we compared the effects of sphingosine and TNFα on PGE_2 production in normal and PKC-deficient cells. Fig. 3 shows that TNFα alone stimulates PGE_2 production equally in control and PKC downregulated cells. As expected, PMA stimulates PGE_2 production in normal cells but not in cells with downregulated PKC activity. Interestingly, the combination of PMA and sphingosine, both of which stimulated some PGE_2 alone, resulted

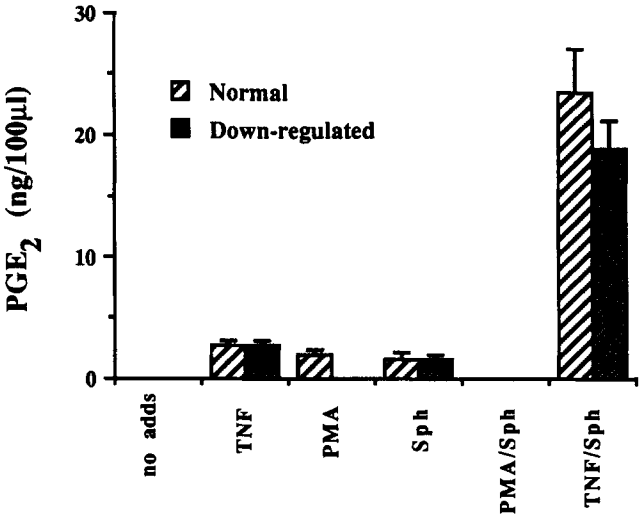

Figure 3. The effect of TNFα, PMA, and sphingosine on PGE_2 production in normal and PKC downregulated fibroblasts. Confluent fibroblast cultures were pretreated with 1 μM PMA for 24 h. Cells were then thoroughly washed and incubated with the following additions for 24 h: PMA, 100 nM, TNFα, 1 ng/ml; sphingosine, 10 μM. PGE_2 was measured by RIA as described in Materials and Methods. The results are expressed as nanograms of PGE_2 produced per 100 μl. Each value represents the mean ± SD of three experiments from identically treated duplicate wells.

in no PGE_2 production, perhaps because of their documented opposing effects on PKC activity. Sphingosine dramatically enhanced TNFα-induced PGE_2 production in normal cells, and this effect was not significantly decreased in cells with downregulated PKC activity, suggesting that in this case sphingosine provides a metabolic signal to the cell that enhances TNFα-mediated PGE_2 production independent of any effects that sphingosine may have on PKC activity.

Specificity of the Sphingosine Effect. To determine the specificity of the stimulatory effect of sphingosine on TNFα-mediated PGE_2 production, we tested the ability of two sphingosine analogues, stearoyl-D-sphingosine and stearylamine, to modulate TNFα-mediated PGE_2 production. Stearoyl-D-sphingosine lacks a free amino group and stearylamine has an alkyl chain length similar to sphingosine, but lacks both hydroxyl groups. Fig. 4 shows that in comparison to the ability of sphingosine to strongly potentiate TNFα-mediated PGE_2 production, stearylamine has some stimulatory effect, and stearoyl-D-sphingosine does not show any synergy with TNFα.

Time Course of Sphingosine-mediated Potentiation of TNFα-induced PGE_2 Production. To examine the temporal relationship between sphingosine treatment and its stimulatory effect on TNFα-induced PGE_2 production, we incubated cells with sphingosine for the times indicated in Fig. 5, washed them with fresh culture media, and then treated them with TNFα (1 ng/ml) for 24 h. Fig. 5 shows that the effect of sphingosine on TNFα-mediated PGE_2 production is extremely rapid. After only a 1-min exposure to sphingosine, TNFα-mediated PGE_2 production was enhanced to 90% of maximal and to 100% after a 5-min exposure. TNFα-mediated PGE_2 production decreased after 1 h preincubation with sphingosine, and longer pre-incubations resulted in progressively lower levels of PGE_2, returning to basal levels after 24 h of exposure to sphingosine. These results indicate that the effects of sphingosine on TNFα-induced PGE_2 production are rapidly manifested and relatively transient. The transient nature of the sphingosine effect may be due to its rapid metabolism to an inactive metabolite by the cell.

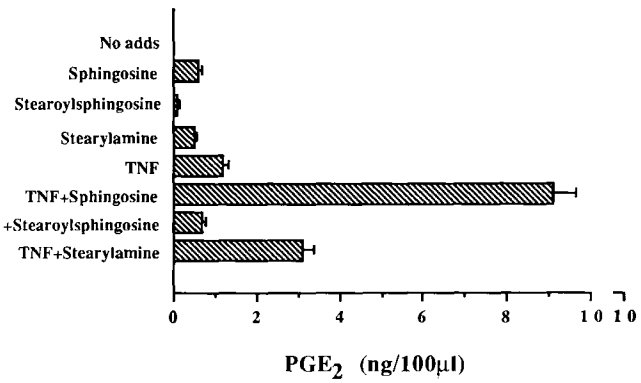

Figure 4. The effect of TNFα, sphingosine, sphingosine analogues, on PGE_2 production. Confluent cultures of human fibroblasts were treated as indicated. After 24 h PGE_2 was measured by RIA as described in Materials and Methods. The results are expressed as nanograms of PGE_2 produced per 100 μl. Each value is the mean ± SD of three experiments from identically treated duplicate wells. The concentration of each addition was: TNFα, 1 ng/ml; sphingosine, 10 μM; stearylamine, 10 μM; stearoyl-D-sphingosine, 10 μM.

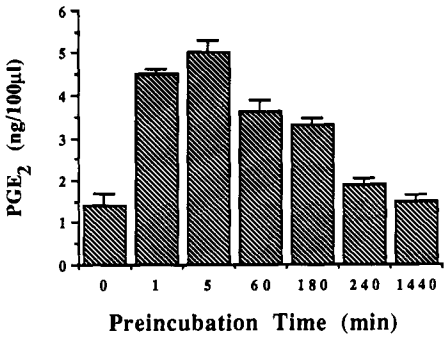

Figure 5. The effect of sphingosine pretreatment on TNFα-mediated PGE_2 production. Confluent fibroblast cultures were exposed to sphingosine (10 μM) for the times indicated. Each well was then extensively washed to remove any remaining sphingosine. Each well was then treated with TNFα (1 ng/ml). Control wells were treated with sphingosine or TNFα alone and with sphingosine/TNFα combination. After 24 h PGE_2 production was measured as described in Materials and Methods. The results are expressed as nanograms of PGE_2 produced per 100 μl. Each value represents the mean of triplicate assays from two separate wells ± SD. PGE_2 production was 0.4 ± 0.14 ng/100 μl in control wells treated with sphingosine alone, 1.4 ± 0.28 ng/100 μl in wells treated with TNFα alone (time zero) and 5.7 ± 0.14 ng/100 μl in wells treated with sphingosine and TNFα at the same time.

The Effect of TNFα and Sphingosine on PLA_2 and Cox Activity in Human Fibroblasts. Cytokine-induced PGE_2 production in human fibroblasts is regulated by the availability of arachidonic acid, released from phospholipids by the action of PLA_2, and on its conversion to PGE_2 by Cox (42). To directly assess the effect of sphingosine and TNFα on PLA_2 and Cox activity, we prepared an extract from sonicated fibroblasts using untreated cells and cells treated for 18 h with TNFα, sphingosine or both. Fig. 6 shows that PLA_2 activity in cells pretreated with sphingosine (10 μM) and/or TNFα (1 ng/ml) is significantly higher than basal PLA_2. Moreover, PLA_2 activity derived from cells pretreated with both sphingosine and TNFα is significantly higher than in cells pretreated with TNFα alone ($p < 0.05$). Similarly, Cox activity is significantly higher in cells treated with sphingosine and TNFα when compared with the activity measured in untreated cells ($p < 0.001$), but not when compared with activity in cells treated with TNFα alone.

Discussion

Sphingolipids have been implicated in modulating many diverse cellular processes, including cellular communication, transformation, proliferation, differentiation, and receptor function (1, 43, 44). The mechanism(s) of action and the signal transduction pathways modulated by these complex lipids remain poorly understood. Although much evidence suggests that sphingosine is a potent inhibitor of PKC activity (1, 13), other studies indicate that sphingosine has bio-

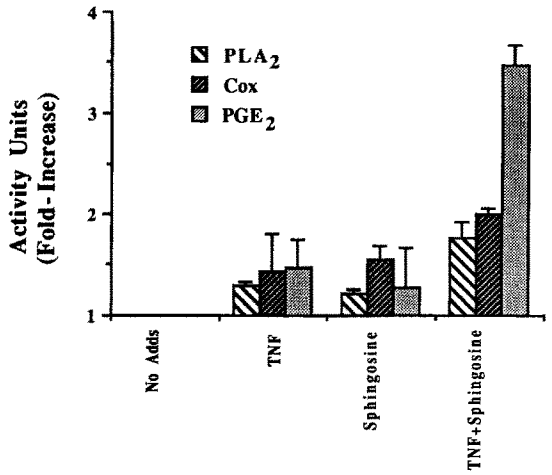

Figure 6. The effect of TNFα and sphingosine on Cox and PLA_2 activities. PLA_2 and Cox activities were measured as described in Materials and Methods. PGE_2 production was measured by RIA. Confluent cultures of human fibroblasts were pretreated with TNFα and/or sphingosine for 18 h before preparation of the supernatant fraction. The concentration of each addition was: TNFα, 1 ng/ml; sphingosine 10 μM. Untreated control PLA_2, Cox, and PGE_2 levels were normalized to 1.0, and the fold stimulation by each treatment was calculated. Each value is the mean of three experiments from two identically treated wells ± SD.

logical effects which are, at least in part, independent of the PKC pathway (4, 5, 16, 18). Thus, caution has been urged in the use of sphingosine as a reagent to inhibit PKC and further, the possibility that sphingosine may not be a physiological modulator of PKC activity must be considered. The findings presented here suggest that sphingosine may be involved in modulating the responsiveness of the cell to TNFα. Sphingosine has also been implicated as a modulator of the actions of epidermal growth factor (4), IL-1 (18), and most likely, other cytokines yet to be identified.

Sphingomyelin turnover involves the hydrolysis of sphingomyelin to ceramide and phosphorylation by a neutral sphingomyelinase. Some have suggested that this is an important biochemical pathway in regulating a variety of cell functions (45). It has been demonstrated recently that TNFα causes an early and reversible sphingomyelin hydrolysis in HL-60 cells accompanied by the concomitant generation of ceramide that subsequently downregulates mRNA production for the *c-myc* protooncogene (28). A variety of other biologically active sphingolipid metabolites, such as sphingosine (4, 18) and sphingosine-1-phosphate (43) are also produced during sphingomyelin turnover. Further, sphingosine may be converted to ceramide or sphingosine-1-phosphate, thus complicating determination of the specific effects of these compounds. Clearly, each of these metabolites may modulate a wide variety of cellular responses including the ability of certain inflammatory cytokines to induce PGE_2 production as shown here.

The ability of TNFα to affect the growth, differentiation, and various other functions of virtually every cell type investigated, appears to be largely determined at the postreceptor level. However, little is known about the precise intracellular pathways which mediate TNFα signal transduction. The effect of TNFα on PKC activation is controversial but several reports suggest that PKC activation may play a major role in TNFα signal transduction in some (24, 25, 34), but not all target cells (26). With respect to transcriptional control, the relationship between TNFα and the activation or repression of specific genes remains to be elucidated. In certain cell types (Jurkat and K562), TNFα activates transcription factor NFkB via a PKC-independent pathway (27), further indicating that TNFα signal transduction does not necessarily require the involvement of PKC.

The fact that TNFα and IL-1 share many functional characteristics (including the stimulation of PGE_2 production) led us to test the effect of sphingosine on TNFα-mediated PGE_2 production in order to determine whether similar mechanisms were involved in TNFα- and IL-1-induced PGE_2 production. Recently we showed that sphingosine greatly potentiates IL-1–mediated PGE_2 production in human fibroblasts independent of PKC activation (18). In fact, the downregulation of PKC activity by prolonged exposure to PMA (40, 41) mimicked the effect of sphingosine, suggesting that active PKC may actually inhibit IL-1-mediated PGE_2 production, and that the stimulatory effect of sphingosine on IL-1-mediated PGE_2 production was due in part to its ability to inhibit PKC activity. Conversely, with respect to sphingosine and TNFα, the inhibition of PKC activity by prolonged exposure to PMA had no effect on TNFα-mediated PGE_2 production in the presence or absence of sphingosine. These findings clearly suggest that some factor, or factors, not involving the PKC pathway, are responsible for the potentiation of TNF-α-mediated PGE_2 production by sphingosine.

The synergistic behavior of sphingosine and TNFα on PGE_2 production appears to be independent of the potential effects of TNFα-induced IL-1 synthesis. Anti-IL-1α, and anti-IL-1β antibodies and IRAP did not inhibit TNFα-induced PGE_2 production or the stimulatory effect of sphingosine, yet they completely blocked the effects of exogenously-added IL-1. Together, these results support the conclusion that TNFα acts independently of TNFα-mediated IL-1 production, not only because the effects of sphingosine and TNFα are manifested quite rapidly but because, as previously shown, the time course of IL-1–mediated PGE_2 production in cells preincubated with sphingosine (18) differs significantly from that of TNFα. Whereas sphingosine exhibits a rapid, transient effect on TNFα-induced PGE_2 production, its effect on IL-1 mediated PGE_2 production was less rapid, but more sustained. This difference may be because although TNFα seems to act entirely independently of PKC, IL-1 mediated PGE_2 production may be downregulated by active PKC, and the inhibition of PKC by sphingosine requires a longer period of time to achieve (18). Clearly, sphingosine potentiates PGE_2 production in response to TNFα or IL-1. However, the mechanism of sphingosine action appears to differ depending upon the external stimulus. Thus, the effects of sphingosine on cytokine-mediated PGE_2 production are complex and cannot be attributed to any single biochemical event or process.

The precise mechanism by which sphingosine potentiates

TNFα-induced PGE_2 production remains unclear. Previous data obtained in studies using IL-1- and sphingosine-treated fibroblasts showed that PLA_2 activity derived from these cells was significantly higher than that detected in extracts obtained from cells treated with IL-1 or sphingosine alone (18). Recently, it has been reported that both TNFα and IL-1 increase PLA_2 mRNA levels and secretion of PLA_2 from the cell (46). We show here that TNFα and sphingosine significantly increase endogenous PLA_2 activity over TNFα levels. While IL-1 has been shown to induce the synthesis of Cox (42), similar studies using TNFα have not been reported. The data presented here indicate that TNFα alone significantly increases Cox activity compared to control levels but sphingosine only slightly enhances the TNFα effect on Cox activity. On the basis of observations such as these, and because increased PGE_2 production is likely to be dependent upon increased PLA_2 and Cox activities, we hypothesize that TNFα and sphingosine synergistically stimulate PGE_2 production in human fibroblasts, at least in part, by increasing arachidonic acid mobilization via TNFα and sphingosine-enhanced PLA_2 activity, providing more substrate (arachidonic acid) for conversion to PGE_2 by Cox. Even slight increases in PLA_2 and/or Cox activity could account for the observed increase in PGE_2 production in response to TNFα and sphingosine because end product (PGE_2) is allowed to accumulate over a relatively long period of time (24 h).

The finding that sphingosine dramatically potentiates TNFα-mediated PGE_2 production could be very important relative to our understanding of the pathophysiologic mechanisms that mediate a variety of inflammatory processes. The role of PGE_2, and other prostanoids, in inflammation is well established and its effects are known to include the mediation of pain, vasodilation, increased vascular permeability, and alterations in immune cell functions. Because sphingosine, and perhaps other sphingolipid metabolites, can so profoundly enhance cytokine-induced PGE_2 production, it is interesting to speculate that the elevated levels of PG production commonly associated with inflammatory disorders such as rheumatoid arthritis may not result from the overproduction of inflammatory cytokines by mononuclear cells, but rather, from increased sphingomyelin turnover in the target cell which can dramatically potentiate the response of the cell to otherwise normal levels of cytokine.

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