Ceramide generation by stimulated sphingomyelinase activity has been implicated in tumor necrosis factor α (TNF) signaling of apoptosis and differentiation. We examined the role of ceramide in a major action of TNF: the initiation of inflammatory events. Sphingomyelinase C at high levels induced inflammatory protein expression in endothelial cells resulting in leukocyte adhesion, but the pattern of induction of adhesion molecules (E-selectin, ICAM-1, VCAM-1) and cytokines (interleukins 6 and 8) differed from that induced by TNF. TNF induced only a small increase in ceramide: using lower doses of sphingomyelinase to mimic this we found that small amounts of ceramide did not induce protein expression, but still rapidly activated Raf-1, mitogen-activated protein/extracellular regulated kinase (ERK) kinase (MEK) and ERKs. TNF additionally caused rapid p38 and JNK mitogen-activated protein kinase activation and efficient NF-κB translocation, which could not be achieved even by high levels of ceramide. Thus activation of the ERK cascade alone is an incomplete endothelial cell stimulus, and the TNF receptor generates at least two signals: Raf-1 activation, which could be ceramide-dependent; and ceramide-independent efficient NF-κB translocation and activation of p38 and JNK mitogen-activated kinases.

Tumor necrosis factor α (TNF) signaling has been examined in the context of cell differentiation, apoptosis, and cell growth, but in vivo TNF is a mediator of systemic inflammation and immune responses (1, 2). A major site of action of TNF for these effects is the vascular endothelium (3), where it induces inflammatory responses by enhancing adhesion molecule expression and cytokine secretion. The binding of TNF to the TNFR1 receptor on endothelial cells (4, 5) is followed by rapid translocation of the transcription factor NF-κB from the cytoplasm to the nucleus (6). This translocation is important for stimulated transcription of the adhesion molecules E-selectin, ICAM-1, and VCAM-1, and the cytokines IL-6 and IL-8 (7), which all possess NF-κB promoter/enhancer elements. The regulated expression of these genes initiates the inflammatory cascade: E-selectin mediates the initial interaction of leukocytes with endothelium (8), while adherent leukocytes are activated by IL-8 to bind ICAM-1 and other ligands on the endothelial surface. The coordinated expression (9) of these molecules by activated endothelial cells results in tight leukocyte adhesion followed by their transmigration into tissue spaces.

The signaling pathways that couple TNF receptor activation to functional responses have remained elusive. TNF activates Raf kinase, initiating the ERK MAP kinase cascade, in some cells, while in others it activates a parallel path(s), likely via MEK kinase, that activates the p38 and JNK MAP kinases (10). How these cascades are initiated remains unknown, but recent data suggest ceramide may couple the TNF receptor to certain downstream events (11-13). TNF activates a sphingomyelinase activity in broken cell preparations (14), implying that ceramide acts high in the signaling cascade, and ceramide in some (15, 16), but not all (17, 18), cell-free systems leads to IκB degradation and NF-κB activation. Since TNF transiently increases cellular ceramide, and increased ceramide levels activate ERK (19, 20) and JNK (20) MAP kinases and NF-κB translocation (21), ceramide is an attractive candidate as an early, essential component of TNF signal transduction.

This outline, however, has been derived only in the context of growth regulation or induction of apoptosis (22). As different domains of the TNF receptor signal apoptosis (23) and transcription factor activation (24, 25), coupling of the receptor to inflammatory events may differ from cell cycle related events. Here we determined if the ceramide proposed in the context of cell growth and viability also underlies the inflammatory effect of TNF on one of its major in vivo targets. We report that ceramide at high levels does activate primary cultures of human endothelial cells to synthesize certain inflammatory proteins. However, the small amount of ceramide evoked by TNF was incapable of stimulating a functional response from these cells, and TNF activated MAP kinase cascades that were inaccessible to ceramide. Conversely ceramide at the low levels induced by TNF rapidly stimulates a Raf-1/ERK kinase cascade, and so may couple TNF receptor activation to this pathway.

**EXPERIMENTAL PROCEDURES**

Materials—Reagents and their sources were as follows: recombinant human TNF, Genentech (San Francisco, CA); Staphylococcus aureus sphingomyelinase C, Sigma Immunochemicals (St. Louis, MO); HBSS and M199, Whittaker Bioproducts (Walkersville, MD); 35-mm multiwell plates, Costar Data Packaging Corp. (Cambridge, MA); hu-
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Ceramide in Endothelial Cells—First we determined if ceramide elicited an inflammatory response in endothelial cells by exposing these cells to the water-soluble synthetic ceramide analog N-octanoylsphinogosine (C8-ceramide). Synthesis of ceramides activated endothelial cells to bind quiescent PMN and, like the response to TNF, this could be inhibited by a blocking monoclonal antibody against E-selectin (Fig. 1). Since this adhesion molecule is solely expressed by activated endothelial cells (34), the synthetic ceramide acted on endothelial cells and not on PMN. Accordingly an anti-β2 integrin antibody, which blocks adhesion of activated PMN, failed to inhibit this endothelial cell-dependent adhesion (data not shown). This stimulation also was not due to contaminating endotoxin (a potent endothelial cell agonist), as polymyxin B failed to block it (data not shown).

Next we determined whether endogenous ceramide, derived from cellular sphingomyelin, also affected this response. Treatment of endothelial cells with sphingomyelin-specific sphingomyelinas C activated them to bind quiescent PMN, and increased amounts of sphingomyelinase C gave enhanced levels of adhesion (Fig. 2A). The response was sigmoidal with no apparent effect at 0.1 unit of enzyme activity, an important issue in light of the results presented below. Again, polymyxin B had no effect on PMN adhesion (data not shown). Increased adhesion was not immediate, but developed over the same period of several hours required for TNF's effects to become manifest (data not shown). We therefore assessed E-selectin surface expression by flow cytometry and found sphingomyelinase

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nase C, like TNF, activated endothelial cells from a basal state where no E-selectin was expressed to one where significant amounts were present on the surface (Fig. 2C). The amount of E-selectin induced by sphingomyelinase C treatment was always less than that expressed in response to TNF, and since this pattern obtained after 4 and 8 h of exposure, it was not due to a markedly different rate of E-selectin expression. Nevertheless, this amount of E-selectin was sufficient to account for the increase in PMN adhesion. A blocking E-selectin monoclonal antibody significantly inhibited TNF-induced adhesion and completely blocked that induced by sphingomyelinase C (Fig. 2B). Thus hydrolysis of cellular sphingomyelin to ceramide is an endothelial cell stimulus that induces at least one key inflammatory response of these cells.

Sphingomyelinase C Does Not Reproduce All of the Effects of TNF—The hypothesis that TNF signaling proceeds via an activated sphingomyelinase C predicts the ceramide produced by this enzyme should exactly mimic responses evoked by TNF. Accordingly, we examined other endothelial cell responses and found sphingomyelinase C induced expression of the adhesion molecules VCAM-1, ICAM-1, as well as E-selectin, and that it induced secretion of the cytokines IL-6 and IL-8 (Fig. 3). However, except for IL-6 expression, even a maximally effective amount of sphingomyelinase C was a weak agonist compared to TNF. Similarly water-soluble ceramide analogs induced expression of all these molecules, but also were weak agonists for these responses (data not shown.) Thus sphingomyelinase C stimulates endothelial cell inflammatory responses, but it appears unlikely that ceramide alone accounts for all the effects of TNF on endothelial cells.

The results above suggest the 55-kDa TNF receptor (4) must, at a minimum, activate another signaling pathway in this cell type. There is a caveat to this conclusion, however, as it is postulated that intracellular pools of sphingomyelin generate the ceramide that mediates signaling (21, 25, 35), and so extracellular sphingomyelinase C might generate ceramide that may not have adequate access to its signaling effector. To circumvent this limitation, we reversibly permeabilized endothelial cells with glass beads under conditions where molecules as large as antibodies gain access to the interior of the cell (28). Permeabilized monolayers were treated with sphingomyelinase C, a water-soluble ceramide analog, or TNF and allowed to reseal. We found permeabilized and resealed endothelial cells still responded to TNF, as they synthesized E-selectin at levels indistinguishable from untreated monolayers, and that this procedure had only a minor stimulatory effect on monolayers exposed only to buffer (Fig. 4). However, when sphingomyelinase C and ceramide from the external aspect of the plasma membrane, or water-soluble ceramide analogs, had access to the interior of the cell, they remained weak agonists for E-selectin expression. We found (Fig. 4) that intracellular ceramide, derived from the cell's own metabolically active pool, also failed to generate significant E-selectin synthesis by using 1-phenyl-2-decanoylamino-3-morpholino-1-propanol to block ceramide metabolism to glycosylceramide. We conclude the failure of ceramide to act as a full inflammatory agonist was not due to inadequate access to the cell's signaling machinery.

TNF Induces a Weak Ceramide Cycle in Endothelial Cells—TNF stimulation can lead to enhanced levels of ceramide through a receptor-stimulated sphingomyelinase(s) activity (14); resynthesis of sphingomyelin completes the ceramide cycle (11). We determined whether this also occurred in endothelial cells, and found that TNF caused a rapid loss of half of the sphingomyelin pool (Fig. 5A). This depressed level was maintained for 1 h before returning to near basal level by 2 h. TNF correspondingly caused a small increase in ceramide levels within 10 min that continued to increase over the 2 h of the experiment (Fig. 5B). However, the increase was only 20% over basal levels by 30 min, with just an 80% increase following 2 h of TNF exposure. Since the loss of sphingomyelin was greater than the accumulation of ceramide, and the timing of sphingomyelin loss and ceramide accumulation differ, we infer much of

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**FIG. 1.** Synthetic ceramide induces E-selectin-dependent PMN adhesion. Endothelial cells were incubated with buffer or 10 μM C8-ceramide for 4 h, and the monolayers were washed and then incubated with buffer or 10 μg/ml anti-E-selectin monoclonal antibody P6E2 for 30 min. PMN adhesion was determined from microscopic images as described under "Experimental Procedures." A, unstimulated, control incubation. B, unstimulated, anti-E-selectin monoclonal antibody. C, C8-ceramide-treated monolayers, control incubation. D, C8-ceramide-treated monolayers, anti-E-selectin monoclonal antibody.
the initial burst of ceramide was metabolized. We also found (Fig. 5B) that TNF did not cause diacylglycerol, an activator of protein kinase C and acidic sphingomyelinase activity (21, 36), to accumulate.

In contrast to TNF, endothelial cells treated with sphingomyelinase C demonstrated a rapid decrease in sphingomyelin levels, equivalent to that induced by TNF treatment, and this depressed level of sphingomyelin content was maintained over the 2 h of the experiment (Fig. 5A). This resulted in a rapid, and large, enhancement in the cellular complement of ceramide (Fig. 5C); within 10 min of exposure to exogenous sphingomyelinase C, there was a sustained rise in cellular ceramide to 9 times its basal level. Despite the large increases in cellular ceramide content, there was not a concomitant change in diacylglyceride levels, demonstrating the substrate specificity of this enzyme. We also determined the amount of cell-associated ceramide following treatment of endothelial cell monolayers with a water-soluble ceramide analog and found a 4–5-fold increase in ceramide levels, with no increase in diglyceride levels or decrement in sphingomyelin content (data not shown). We conclude that TNF induces a complete ceramide cycle in endothelial cells, but that the peak ceramide accumulation is far less than that induced by exogenous sphingomyelinase C. Additionally, TNF-induced ceramide accumulation relevant to early signaling events was modest, being only 20–30% over basal levels.

We determined if a small increase in ceramide could mimic TNF by reducing the amount of sphingomyelinase C in our experiments. We found a graded increase in cellular ceramide could be attained by varying the amount of sphingomyelinase C (Fig. 6A). Although this did not occur in a linear fashion, 0.1 unit of enzyme produced somewhat more ceramide accumulation than did TNF treatment. We then examined E-selectin accumulation as a function of sphingomyelinase C activity and found (Fig. 6B) that 0.5 and 1 unit gave levels of expression...
that were about half that induced by TNF. However, E-selectin expression was completely undetectable by this sensitive assay when the cells were exposed to 0.1 unit of sphingomyelinase C. Thus the small alterations in ceramide content induced by TNF alone are not able to stimulate E-selectin accumulation.

TNF Activates Both Raf/ERK and "Stress" JNK, p38 Kinase Cascades—We sought to determine by what mechanisms TNF and ceramide activate endothelial cells, and to elucidate the differences between them. TNF signaling is complicated in that in some systems it is Raf-dependent (24) while in others it is Raf-independent (37–39). Similarly, ceramide leads to Raf (24) and ERK (19) activation in the "growth" pathway (see Fig. 10) in some cells, but is part of the "stress" pathway leading to JNK activation (40, 41) in others. First, we determined whether, and at what level, ceramide acts in the endothelial cell kinase cascade by determining Raf activity in immunoprecipitates of endothelial cells treated with TNF or sphingomyelinase. We found that ceramide acted high in the kinase cascade as it maximally activated Raf-1 (Fig. 7). Additionally, ceramide was a potent agonist for Raf activation as cell treated with 0.1 unit of sphingomyelinase C demonstrated a significant increase in Raf-1 activity (Fig. 7). Thus, even a low level of ceramide, such as generated upon TNF stimulation, is a rapid and effective stimulus for Raf-1 activation in endothelial cells.

We determined if Raf-1 activation stimulated downstream MAP kinase activities by two approaches; we examined differences in their mobility during SDS-polyacrylamide electrophoresis, and we directly assayed kinase activity by immunoprecipitation and phosphorylation of preferred substrates. Western blot analysis shows (Fig. 8A) both p42 (ERK1) and p44 (ERK2) MAP kinases have altered electrophoretic mobility, consistent with enhanced phosphorylation, in PMA-, TNF-, and sphingomyelinase C-treated cells. For the latter agonists, the mobility shift was dependent on the time of stimulation, with changes occurring as early as 5 min after agonist addition. In
contrast, a rapid alteration in the mobility of p38 and JNK-1 was detected only in TNF-treated cells; for at least the first 15 min after ceramide exposure, these two MAP kinases did not demonstrate the band broadening associated with an enhanced phosphorylation state. PMA behaved like sphingomyelinase C in that it specifically activated ERK1 and ERK2, but failed to induce p38 or JNK phosphorylation.

We confirmed this surrogate assay reflected enzyme activation by determining kinase activity in immunoprecipitates of cell lysates, and we found that ERK activity increased with time of exposure to either TNF or sphingomyelinase C (Fig. 8B). Additionally, we found even 0.1 unit of sphingomyelinase C was a maximally effective stimulus for ERK activity (Fig. 8C). We then assayed p38 and JNK-1 activity to find that these two MAP kinases were activated in a time-dependent fashion following TNF treatment. Again, even the large amounts of ceramide generated by 1 unit of sphingomyelinase C failed to quickly activate either of these kinases. Since TNF stimulates all three MAP kinases, while ceramide effectively activates only ERK1 and ERK2, the TNF receptor initiates signaling events leading to activation of these other MAP kinases in a ceramide-independent fashion.

TNF-generated Ceramide Is Suboptimal for NF-κB Translocation—The ability to selectively activate the Raf/ERK pathway with low levels of ceramide offers the opportunity to determine if this cascade can solely be responsible for translocation of NF-κB to the nucleus, or if other TNF-initiated events account for this. The results of a electrophoretic mobility shift assay (Fig. 9) show that the nuclei of quiescent endothelial cells contained a small amount of constitutive NF-κB-like binding activity. Exposure of monolayers to TNF for 60 min alters this such that nuclei now contained large amounts of the specific p65-p50-like band. Formation of this complex was completely inhibited by excess unlabeled NF-κB oligomers, but not by Oct-1 or AP-1 oligomers (data not shown). Exogenous sphingomyelinase C also induced significant nuclear NF-κB accumulation, reaching approximately half that of TNF exposed cells, but this occurred only at high sphingomyelinase C levels. Similar results were obtained by immunolocalization of the p65 NF-κB subunit (data not shown). When the amount of sphingomyelinase C was adjusted to 0.1 unit/ml, the level of NF-κB binding activity was very much less than that of TNF-exposed cells. Thus ceramide at high levels can induce NF-κB translocation, consistent with its pro-inflammatory effect, but the modest ceramide accumulation in response to TNF does not account for this.

DISCUSSION

Ceramide has received attention as a possible link between the TNF receptor and subsequent functional responses, although the relevant ceramide-stimulated activity or its downstream effector(s) have yet to be determined. Also undeter-
The growth factor-induced pathway results in activation of NF-κB and the expression of functional protein as the readout. We asked whether ceramide was an agonist in these cells for inflammatory responses and whether it could participate in the TNF signal transduction pathway. Ceramide, either as a synthetic analog or produced by exogenous sphingomyelinase, activated the inflammatory response of primary cultures of human endothelial cells. Ceramide caused endothelial cells to bind human PMN, the first step in physiologic inflammation, through the synthesis and expression of E-selectin. Additionally, ceramide induced endothelial cell expression of IL-8 that activates neutrophils and other cells involved in an acute inflammatory response. We find a similar group of responses following exposure of endothelial cells to the sphingomyelinase D of the brown recluse spider or Corynebacterium pseudotuberculosis (29). Ceramide can therefore act as an inflammatory stimulus in vitro and in vivo, but this occurs only after an unphysiologic increase in cellular ceramide content.

We next investigated whether ceramide could have a role in TNF signaling in this target cell. We documented that TNF induced a sphingomyelin cycle in endothelial cells, but found TNF enhanced ceramide levels only 20–30% over basal levels during the first 30 min of stimulation. This was less than the enhancement caused by 0.1 unit/ml sphingomyelinase C, an amount of enzyme that was not a stimulus for E-selectin expression or PMN adhesion. From this observation, and the fact that even high levels of ceramide accumulation failed to result in significant expression of VCAM-1 and ICAM-1, we conclude ceramide does not solely account for TNF activation of the endothelial cell inflammatory response. Furthermore, our results suggest caution in interpreting other data as the use of exogenous sphingomyelinase or ceramide analogs at inappro-
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NF-\(\kappa\)B translocation to the nucleus following I kB phosphorylation and degradation (46). Translocation of the p65 subunit (data not shown) and formation of functional p65-p50 like complexes in the nuclei of endothelial cells occurred in response to both TNF and high levels of sphingomyelinase C. However, ceramide at the level generated by TNF was suboptimal for efficient NF-\(\kappa\)B translocation, so activation of Raf-1 alone leading to activation of ERK-1 and 2 is not sufficient for this response, as found in a murine pre-B cell line (24). It is apparent ceramide-independent TNF effector mechanisms promote the bulk of NF-\(\kappa\)B translocation; while this might result from cooperative signals derived from p38 and JNKK-1 MAP kinase activation, higher levels of ceramide, which still failed to activate these MAP kinases, were able to overcome the missing event(s). These data show a variety of signals from the TNF receptor are needed for the induction of an effective inflammatory response from endothelial cells, and that ceramide is only one of them.

MAP kinase activation, transcription factor phosphorylation, and NF-\(\kappa\)B translocation lead to endothelial cell gene expression of E-selectin, ICAM-1, VCAM-1, IL-6, and IL-8. In all these transcriptionally regulated genes, NF-\(\kappa\)B cooperatively interacts with other transcription factors at the promoter regions to induce gene transcription (7, 47). In the case of TNF induction of E-selectin, phosphorylation of ATF-2 and c-j un heterodimers by p38 and JNKK-1 activate transcription in conjunction with NF-\(\kappa\)B (47, 48). Ceramide, however, fails to activate the stress-activated MAP kinase pathways, fails to phosphorylate ATF-2 and c-j un, and is a poor agonist for E-selectin expression. IL-6 and IL-8 are regulated transcriptionally by ERK-induced phosphorylation of C/EBP-\(\beta\), in conjunction with NF-\(\kappa\)B (49–51), and these are well expressed by both TNF and sphingomyelinase C. These factors were, however, insufficient for transcriptional up-regulation of VCAM-1 and ICAM-1. The use of ceramide as a receptor-independent agonist thus reveals the complexity of signal integration at the promoter region in a coordinated inflammatory response.

In conclusion, a role for ceramide in the expression of inflammatory gene products has been inferred from the ability of significant increases in cellular ceramide, induced by exogenous sphingomyelinase or water-soluble ceramide analogs, to mimic the effects, or some of the effects (17), of TNF on cell differentiation, induction of apoptosis, and message accumulation from reporter gene constructs. However, such correlative conclusions can be misleading if significant differences in ceramide accumulation occur following the two types of stimulation. Ceramide alone, but only at high levels, can induce disjointed inflammatory responses, but this is likely only relevant to bacterial sepsis by prokaryotes (e.g. Clostridium perfringens, Staphylococcus aureus, and Bacillus cereus) that express this activity as an exotoxin (52), or following envenomation by the brown recluse spider (29). Identification of a selective Raf/ERK-specific agonist, at least in this cell type, allowed us to demonstrate that modest, and early, increases in cellular ceramide following TNF treatment can activate Ras growth factor receptor-stimulated pathway. Activation of this cascade coincides with small increase in NF-\(\kappa\)B translocation to the nucleus, but this alone is not sufficient to induce an inflammatory response. The TNF receptor also stimulates the stress-activated p38 and JNK-1 kinase cascades, in a ceramide-independent fashion, and this or another ceramide-independent signal(s) activates efficient NF-\(\kappa\)B translocation required for induction of the inflammatory response by endothelial cells.

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REFERENCES

1. Rothe, J., Lesslauer, W., Lottcher, H., Lang, Y., Koeble, P., Kontgen, F., Althage, A., Zinkernagel, R., Steinmetz, M., and Bluethmann, H. (1993) Nature 364, 798–802
2. Pfeffer, K., Matsuyama, T., Kundig, T. M., Wakeham, A., Kishihara, K., Shahinian, A., Wiegmann, K., Ohashi, P. S., Kronke, M., and Mak, T. W. (1993) Cell 73, 457–467
3. Pober, J. S., and Cutran, R. S. (1993) Physiol. Rev. 70, 427–451
4. Slowik, M. R., DeLucia, L. G., Fiers, W., and Pober, J. S. (1993) Am. J. Pathol. 143, 1724–1730
5. MacKay, F., Lottcher, H., Stueber, D., Gerh, G., and Lesslauer, W. (1993) J. Exp. Med. 177, 1277–1286
6. Keller, R. A., and Kronke, M. (1994) J. Cell Biol. 126, 5–9
7. Collins, T. (1995) Lab. Invest. 68, 499–508
8. McEver, R. P., Moore, K. L., and Cummings, R. D. (1995) J. Biol. Chem. 270, 11023–11028
9. Zimmerman, G. A., Lorant, D. E., McIntyre, T. M., and Prescott, S. M. (1993) J. Exp. Med. 177, 1277–1286
10. Cant, E., and Mahadevan, L. C. (1993) Trends Biochem. Sci. 20, 117–123
11. Hannun, Y. A. (1994) J. Biol. Chem. 269, 3125–3128
Ceramide in Endothelial Cell TNF Kinase Cascades

12. Kolesnick, R., and Golde, D. W. (1994) Cell 77, 325–328
13. Cruz, R., Sarcich, J. L., Tomasselli, A. G., and Manning, A. M. (1995) FASEB J. 9, A127
14. Dresel, K. A., Mathias, S., and Kolesnick, R. N. (1992) Science 255, 1715–1718
15. Reddy, S. A. G., Chaturvedi, M., Darnay, B. G., Chan, H., Higuchi, M., and Aggarwal, B. B. (1994) J. Biol. Chem. 269, 25369–25372
16. Machleidt, T., Wiegmann, K., Henkel, T., Schutze, S., Baueuerle, P., and Kronke, M. (1994) J. Biol. Chem. 269, 13760–13765
17. Dbaibo, G., Obeid, L. M., and Hannun, Y. A. (1993) J. Biol. Chem. 268, 17751–17757
18. Betts, J. C., Agranoff, A. B., Nabel, G. J., and Shayman, J. A. (1994) Science 265, 1412–1416
19. Raines, M. A., Kolesnick, R. N., and Golde, D. W. (1993) J. Biol. Chem. 268, 14572–14575
20. Saleem, A., Yuan, Z. M., Taneja, N., Rubin, E., Kufe, D. W., and Kharbanda, R. J. (1994) J. Biol. Chem. 269, 26121–26127
21. DeWitt, D. L., and Smith, W. L. (1988) Science 239, 911–917
22. Pushkareva, M., Obeid, L. M., and Hannun, Y. A. (1995) J. Biol. Chem. 270, 22689–22692
23. Tartaglia, L. A., Ayres, T. M., Wong, G. H. W., and Goeddel, D. V. (1993) J. Biol. Chem. 268, 765–776
24. Belka, C., Wiegmann, K., Adam, D., Holland, R., Neuloh, M., Herrmann, F., Kronke, M. (1992) J. Biol. Chem. 267, 1715–1718
25. Zimmerman, G. A., McIntyre, T. M., and Prescott, S. M. (1995) J. Biol. Chem. 270, 22730–23537
26. Zimmerman, G. A., McIntyre, T. M., and Prescott, S. M. (1990) J. Clin. Invest. 80, 529–540
27. Fennell, D. F., Whatley, R. E., McIntyre, T. M., Prescott, S. M., and Zimmerman, G. A. (1991) Arterioscler. Thromb. 11, 97–106
28. Patel, K. D., Modur, V., Zimmerman, G. A., Prescott, S. M., and McIntyre, T. M. (1994) J. Clin. Invest. 94, 631–642
29. Laemmli, U. K. (1970) Nature 227, 680–685
30. Wehrich, A. S., McIntyre, T. M., McEver, R. P., Prescott, S. M., and Zimmerman, G. A. (1995) J. Clin. Invest. 95, 2297–2303
31. Read, M. A., Whitely, M. Z., Williams, A. J., and Collins, T. (1994) J. Exp. Med. 178, 503–512
32. Bligh, E. G., and Dyer, W. J. (1959) Can. J. Biochem. Physiol. 37, 911–917
33. Bligh, E. G., and Dyer, W. J. (1959) Can. J. Biochem. Physiol. 37, 911–917
34. Bevilacqua, M. P., and Nelson, R. M. (1993) J. Clin. Invest. 91, 379–387
35. Linardic, C. M., and Hannun, Y. A. (1994) J. Biol. Chem. 269, 23530–23537
36. Schutze, S., Potthoff, K., Machleidt, T., Berkovic, D., Wiegmann, K., and Kronke, M. (1992) Science 255, 765–776
37. Westwick, J. K., Witzel, C., Minden, A., Karin, M., and Brenner, D. A. (1994) J. Biol. Chem. 269, 26396–26401
38. Winston, B. W., Lange-Carter, C. A., Gardner, A. M., Johnson, G. L., and Riches, D. W. H. (1995) Proc. Natl. Acad. Sci. U.S.A. 92, 1614–1618
39. Minden, A., Lin, A., Lange-Carter, C., Derijard, B., Davis, R. J., Johnson, G. L., Karin, M., and McMahon, M. (1994) Science 266, 1719–1723
40. Kyriakis, J. M., Banerjee, P., Nikolakaki, E., Dai, T., Rubie, E. A., Ahmad, M. F., Avruch, J., and Woodgett, J. R. (1994) Nature 369, 156–160
41. Westwick, J. K., Bielaikwa, A. E., Dbaibo, G., Hannun, Y. A., and Kronke, M. (1992) J. Biol. Chem. 267, 17997–18001
42. Wiegmann, K., Schutze, S., Kampen, E., Himmler, A., Machleidt, T., and Kronke, M. (1992) J. Biol. Chem. 267, 17997–18001
43. Andrews, N., Salavayre, R., and Lavede, T. (1994) Biochem. J. 303, 341–345
44. Olivera, A., Buckley, N. E., and Spiegel, S. (1992) J. Biol. Chem. 267, 26121–26127
45. Derijard, B., Raingeaud, J., Barrett, T. W., U-H. Han, J., Ulevitch, R. J., and Davis, R. J. (1996) Science 274, 682–684
46. Brown, K., Gerstberger, S., Carlson, L., Franzoso, G., and Siebenlist, U. (1995) Science 267, 1485–1488
47. Collins, T., Read, M. A., Neish, A. S., Whitely, M. Z., Thuo, D., and Mianatis, T. (1995) FASEB J. 9, 899–909
48. Kaszubowska, W., van Hulsduijnen, R. H., Ghera, P., DeRanay-Schenk, A.-M., Chen, B. P. C., Hui, T., Delamar, J. F., and Welsman, J. (1993) Mol. Cell. Biol. 13, 7180–7190
49. Matsusaka, T., Fujikawa, K., Nishio, Y., Mukaida, N., Matsuoka, K., Kishimoto, T., and Akira, S. (1993) Proc. Natl. Acad. Sci. U.S.A. 90, 10193–10197
50. Stein, B., and Baldwin, A. S., Jr. (1993) Mol. Cell. Biol. 13, 7191–7198
51. Stein, B., Cogswell, P. C., and Baldwin, A. S., Jr. (1993) Mol. Cell. Biol. 13, 3964–3974
52. Mollby, R. (1978) in Bacterial Toxins and Cell Membranes (J. ejaezewicz, J., and Wadstrom, T., eds) pp. 367–424, Academic Press, London
Endothelial Cell Inflammatory Responses to Tumor Necrosis Factor α: CERAMIDE-DEPENDENT AND -INDEPENDENT MITOGEN-ACTIVATED PROTEIN KINASE CASCADES

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