Biochemical and Biophysical Research Communications **263,** 63–67 (1999)
Article ID bbrc.1999.1312, available online at http://www.idealibrary.com on IDEAL®

Ceramide Synergizes with Phorbol Ester or Okadaic Acid to Induce IκB Degradation

Paula C. Fernandez and Dirk A. E. Dobbelaere[1]
Institute of Animal Pathology, Molecular Pathology, University of Berne, Länggass-strasse 122, CH-3012 Berne, Switzerland

Received July 20, 1999

Ceramide is a lipid second messenger which is generated in response to stimulation of a number of surface receptors, treatment with chemotherapeutic agents, or ionising radiation. Depending on the target cell, ceramide induces diverse biological responses including apoptosis, cell-cycle arrest, differentiation, and also proliferation. We studied the effect of ceramide on the degradation of IκB, the cytoplasmic inhibitor of the transcription factor NF-κB. We show that ceramide treatment results in reduced levels of phosphorylated IκBα and degradation of both IκBα and IκBβ. Ceramide synergised with okadaic acid (OA), a compound which interferes with the protein phosphatase 2A-controlled component of the NF-κB activation pathway, enhancing OA-induced IκB degradation. Ceramide also synergised with phorbol 12-myristate 13-acetate, which mimics protein kinase C activation. Finally, we show that the synergistic effect of ceramide with OA or phorbol ester can be observed in primary lymph node T-cells as well as in transformed T-cells.

Ceramide, a product of sphingomyelin hydrolysis, has emerged as a potentially important lipid second messenger (1). Intracellular ceramide levels increase in response to different extracellular stimuli delivered through receptors for TNF, IL-1 or Fas-ligand and by chemotherapeutic agents or ionising irradiation. Biochemical targets include a cytosolic ceramide-activated phosphatase, a membrane-bound serine/threonine protein kinases and Protein kinase C (PKC) ζ which have also been linked to the biological effects induced by ceramide. These include diverse responses such as apoptosis, cell-cycle arrest, differentiation and proliferation (for reviews see (2–4)). Several stimuli that induce the generation of ceramide also activate the transcription factor NF-κB. Activation of NF-κB involves the degradation of its cytoplasmic inhibitors, IκBs, of which IκBα and IκBβ are the most important members (reviewed in (5, 6)). Phosphorylation by specific kinases (summarised in (7)) targets IκB for proteosomal degradation, freeing NF-κB and allowing it to translocate to the nucleus where it binds to cognate DNA sequences (κB sites) in the regulatory region of a large number of genes that are involved in immune responses and inflammation. NF-κB is important in T-cells since it participates in the regulation of IL-2 and IL-2Rα gene expression and also activates human immunodeficiency virus replication. In addition, NF-κB has also been shown to protect cells against apoptosis (8, 9). IL-1 and TNF, two potent activators of NF-κB, stimulate sphingomyelin hydrolysis resulting in ceramide generation (10). The exact role of ceramide in inducing the activation of NF-κB, however, is still uncertain and conflicting opinions exist.

TNF may induce the activation of an acidic sphingomyelinase (aSMase), and it has been proposed that the ceramide that is generated activates NF-κB (10, 11). Exogenous ceramides have for the most part been shown not to induce nuclear translocation of NF-κB, however (12, 13), and stimulation of NF-κB by ceramide is small relative to the induction by TNF. In several reports, ceramide also did not induce transcription of NF-κB-dependent genes (14, 15) and in some reports, the ceramide response could be dissociated from NF-κB activation (12, 13, 16). For instance, ceramide is not detected in cells treated with TNF, until after NF-κB is induced and an inhibitor of aSMase does not inhibit TNF-induced activation of NF-κB. In addition, NF-κB can be induced by TNF in Niemann-Pick fibroblasts that lack acid SMase (17).

Arguments in favour of a role for ceramide in NF-κB activation also exist, however. In T-cells, it has been shown that stimulation of CD28, the receptor for T-cell co-stimulation, activates aSMase, yielding ceramide in both transformed and primary T-cells (18). Overexpression of aSMase in Jurkat T-cell stimulated via the TCR/CD3 complex could replace CD28 activation of

[1] Corresponding author. Fax: +41-31-6312635. E-mail: dirk.dobbelaere@itpa.unibe.ch.

0006-291X/99 $30.00

NF-κB. *In vitro,* IκBα degradation could directly be induced by addition of SMase or synthetic ceramide to a cell-free system and degradation was suppressed by dichloroisocoumarin, suggesting that SMase triggers the degradation through a serine-like protease (19).

NF-κB activation is subject to several levels of control, involving phosphatase and kinase activity. A number of compounds exist that mimic physiological stimuli by interfering with components of the NF-κB activation pathway. This way, NF-κB can be activated by okadaic acid (OA) (20), a potent inhibitor of phosphatase 2A (PP2A). The latter has been shown to be a negative regulator of IκB kinase (21), preventing IκB phosphorylation and subsequent degradation. Phorbol esters such as phorbol 12-myristate 13-acetate (PMA) stimulate PKC by mimicking the effects of diacylglycerol (DAG), the physiological activator of PKC, and also induce NF-κB activation. DAG is derived from phosphatidylinositol and/or phosphatidylcholine and is a pivotal second messenger in T-cell activation, produced upon stimulation of a range of T-cell surface membrane receptors, including the TCR/CD3 complex, the receptors for TNF and IL-1 and the receptor for costimulation, CD28 (22).

Studies on T-cells have shown that ceramide, generated by overexpression of aSMase, can potentiate NF-κB activation induced by other signals such as TCR/CD3 (18) and co-treatment of cells with ceramide and TNF also showed enhancement of NF-κB activation as compared to TNF alone (12). Ceramide thus appears to cooperate with different pathways to induce NF-κB activation, but the nature of the pathways with which ceramide cooperated was not identified. In this paper, we focused on IκB degradation and used membrane-permeable ceramide in combination with OA or phorbol ester to investigate whether cooperation exists between ceramide-, DAG- and PP2A-controlled NF-κB activation pathways. We show that ceramide potentiates OA- or PMA-induced degradation of both IκBα and IκBβ with different kinetics. This effect could be observed in primary lymph node (LN) T-cells as well as in transformed T-cells.

MATERIALS AND METHODS

Cells, cell line. The *T. parva* infected lymphocyte cell line TpM(803) is $CD4^+$, $CD8^-$, α/β TCR^+ (23). LN T-cells were prepared as described previously (24). Cultures were grown at 37°C in Leibovitz L15 medium containing 10% heat-inactivated FCS, 20 mM Hepes, 20 μg/ml L-glutamine, 100 U/ml benzylpenicillin and 100 μg/ml streptomycin sulfate. Cells were initially seeded at 2×10^5/ml (day 0) and passaged on day 3 when cell density exceeded 1×10^6/ml.

Chemicals. Phorbol 12-myristate 13-acetate (PMA) and ionomycin were purchased from Sigma; C_2-ceramide and dihydro-ceramide were purchased from Calbiochem and dissolved in DMSO; okadaic acid (OA) was from LC laboratories.

Cell lysates. Prior to lysis, cells were washed 2× in ice-cold PBS, pH 7.4. All steps were carried out at 4°C. Cells were suspended in NP-40 lysis buffer (50 mM Tris, pH 7.4, 100 mM NaCl, 0.5% NP-40) containing protease and phosphatase inhibitors (1 mM PMSF, 50 mM NaF, 1 mM $NaVO_4$, 10 μg/ml each of leupeptin and aprotinin). Following 15 min incubation on ice, the lysate was cleared by centrifugation at 14,000 × *g* for 15 min. Protein concentrations were determined by the Bradford method. Extracts were either used immediately or stored at −80°C until use.

Immunoblots. Lysates were boiled in Laemmli sample buffer and equal amounts of protein (20 or 30 μg) were electrophoretically separated by PAGE (10%) and transferred to nitrocellulose membranes. For immunoblot analysis, rabbit anti-peptide polyclonal Ab with the following specificities were used: IκBα (dilution 1:500; raised against the C-terminal 21 amino acids; sc-371, Santa Cruz) and IκBβ (dilution 1:500; raised against the C-terminal 20 amino acids; sc-945). Membranes were blocked, washed, and bound primary Ab detected using horseradish peroxidase conjugated goat anti-rabbit IgG (1:5,000; New England Biolabs, Nr. 7071-1) and enhanced chemiluminescence.

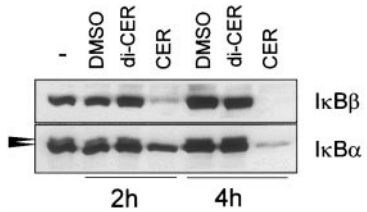

FIG. 1. Ceramide induces IκB degradation in the transformed T-cell line TpM(803). Treatment with ceramide results in reduced levels of phosphorylated IκBα and IκB degradation. TpM(803) T-cells were either left untreated (−) or treated for 2 or 4 h with 100 μM C_2-ceramide (CER) or dihydro-C2-ceramide (di-CER). Whole cell extracts were subjected to immunoblot analysis using antibodies directed against either IκBα (lower panel) or IκBβ (upper panel). Arrowheads indicate the phosphorylated (upper) and unphosphorylated (lower) forms of IκBα. As a control for the solvent in which ceramide was dissolved, cells were also cultured for 4 h in the presence of DMSO (0.1% vol/vol).

RESULTS

In a first set of experiments, we tested whether ceramide affected steady state levels of IκB. In the transformed T-cell line, TpM(803), NF-κB is constitutively activated with both transformation and NF-κB activation being dependent on the presence of the intracellular protozoan parasite *Theileria parva* in the T-cell cytoplasm (25–27). Despite the continuous degradation, permanently high levels of IκBα can be found, which are induced by activated NF-κB, stimulating *de novo* IκBα expression (26). Part of the steady state IκBα in TpM(803) T-cells is phosphorylated; this is reflected by the presence of an IκBα form with reduced electrophoretic mobility in PAGE. Treatment of TpM(803) cells with ceramide for 2 h resulted in the disappearance of phosphorylated IκBα (Fig. 1) and steady state levels of IκBα were markedly reduced after 4 h. Compared to IκBα, levels of IκBβ levels were even more reduced and IκBβ was undetectable after 4 h of treatment. Inactive dihydro-C_2-ceramide, used

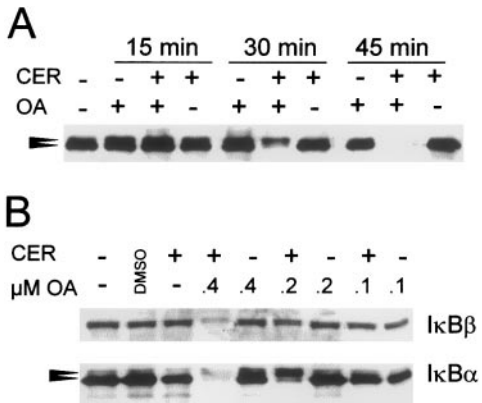

FIG. 2. Synergistic effects of ceramide and OA on the degradation of IκB. (A) Time course experiment showing the synergistic effects of OA and ceramide on IκBα degradation. TpM(803) T-cells were treated for 15, 30, or 45 min with 100 μM C_2-ceramide, 0.4 μM OA, or both together. Immunoblot analysis of whole cell extracts was performed using anti-IκBα antibodies. Arrowheads indicate the phosphorylated and unphosphorylated forms of IκBα. (B) TpM(803) T-cells were cultured for 1 h in the presence or absence of 100 μM C_2-ceramide and increasing doses of OA ranging from 0.1 to 0.4 μM as indicated. Levels of IκBα and IκBβ in cell extracts were examined by immunoblot analysis as described above. Arrowheads indicate the phosphorylated and unphosphorylated forms of IκBα.

as a control, nor the ceramide solvent DMSO interfered with IκB stability.

Next we tested whether ceramide could enhance the IκB degradation induced by two the NF-κB-activators, the PP2A inhibitor OA and PMA, an activator of PKC. Treatment of TpM(803) cells with low doses of OA (0.4 μM) had no, or only marginal effects on IκBα levels. In time course experiments in which TpM(803) cells were treated with OA, ceramide, or a combination of both, IκBα degradation could be detected within 30 min when cells were treated with ceramide and OA together (Fig. 2A and Fig. 3). Interestingly, ceramide clearly did not prevent OA-induced phosphorylation of IκBα since IκBα remaining at 30 min consisted solely of phosphorylated IκBα as judged by its reduced electrophoretic mobility. By 45 min, IκBα had all but disappeared. IκBβ was affected in a similar manner (Fig. 3) with only trace amounts of IκBβ detectable after 30 minutes of combined treatment. Synergy between ceramide and OA was further demonstrated in an OA titration experiment (Fig. 2B). Phosphorylation and degradation of IκBα could be observed in cells exposed to 0.2 μM OA provided ceramide was present. Upon treatment with ceramide and 0.4 μM OA only traces of IκB were detectable, whereas 0.4 μM OA alone had no effect.

The phorbol ester PMA is a potent activator of PKC. Considering that PKC participates in the activation of a wide range of signalling pathways, we tested whether PMA and ceramide also synergise in inducing IκB degradation. TpM(803) cells were treated for 30, 60 or 120 min with PMA in the presence or absence of ceramide (Fig. 3). PMA on its own failed to induce IκB degradation; on the contrary, a moderate increase in IκB levels after 2 h of treatment could repeatedly be observed. As demonstrated in Fig. 1, ceramide alone induced a partial IκBα degradation which was most pronounced after 120 min. In the presence of PMA and ceramide, levels of IκBα and IκBβ were markedly reduced within 30 minutes demonstrating that ceramide and PMA synergise to induce IκB degradation. Interestingly, in contrast to IκBα, IκBβ degradation appeared to be associated with the appearance of additional, most likely phosphorylated, forms of IκBβ. Stimulation by PMA and by PMA combined with ceramide was also carried out in the presence or absence of ionomycin. No enhancement of PMA-induced degradation of IκB could be observed in the presence of ionomycin.

Finally, we determined whether similar synergistic effects could also be observed in primary LN T-cells in which NF-κB is not activated. LN T-cells were stimulated for 30, 60 or 120 min with ceramide, low doses of OA or PMA, or combinations of either of two compounds (Fig. 4). Ceramide alone induced only very limited IκB degradation. Phosphorylated IκBα could readily be detected in OA-treated cells and increased levels IκBα could be detected after 120 min of treatment. IκBα degradation commenced within 30 min of treatment with ceramide and OA together, and no IκBα could be detected at 60 or 120 min. Levels of IκBβ, on the other hand, were not affected by treatment for 30 min with OA and ceramide together. At 60 min, levels of IκBβ were reduced, however, and by 120 min, no more IκBβ could be detected.

IκBα degradation induced by treatment with ceramide together with PMA was markedly slower than that observed for ceramide and OA. IκBβ degradation induced by ceramide and PMA, on the other hand, started within 30 min and IκBβ was hardly detectable after 60 or 120 min.

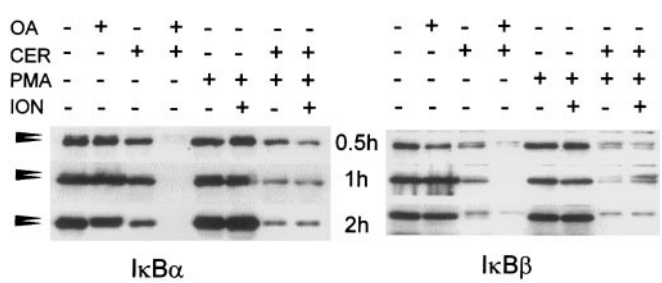

FIG. 3. Synergistic effects of ceramide and phorbol ester on the degradation of IκB. TpM(803) T-cells were treated for 0.5, 1, or 2 h with 100 μM C_2-ceramide, 100 ng/ml PMA, 0.4 μM OA, or 2 μM ionomycin as indicated. Levels of IκB were determined by immunoblot analysis using anti-IκBα (left panel) and IκBβ antibodies (right panel). Arrowheads indicate the phosphorylated and unphosphorylated forms of IκBα.

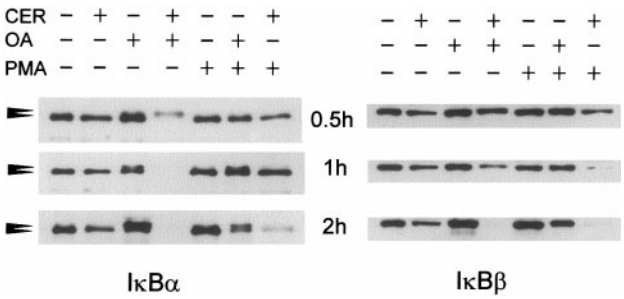

FIG. 4. The synergistic effects of ceramide, OA, and PMA can also be observed in primary LN T-cells. Freshly isolated LN T-cells were cultured for 0.5, 1, or 2 h in the presence of 100 μM C_2-ceramide, 0.4 μM OA, or 100 ng/ml PMA as indicated. Cell extracts were analysed by immunoblot for levels of IκBα (left) and IκBβ (right). Arrowheads indicate the phosphorylated and unphosphorylated forms of IκBα.

DISCUSSION

Some studies suggest an essential role for ceramide, generated upon activation of aSMase, in TNF- or IL-1-mediated NF-κB activation (10, 11, 28). Other studies, however, provide evidence against a role for ceramide in the NF-κB activation pathway or report that ceramide on its own contributes only minimally to NF-κB activation (12, 13, 16, 29). Our observations show that ceramide can act in concert with at least two other stimuli to induce IκB degradation in T lymphocytes. One of these stimuli, induced by PMA, results in the activation of PKC which mediates cellular responses to a broad range of signals, including NF-κB activation (20). The other involves the induction of IκB degradation through OA-mediated inhibition of a PP2A, which regulates the activation of IκB kinases (21).

When cells were treated with C2-ceramide alone, IκB degradation could only be observed after prolonged exposure and when high doses were added to the culture medium. This is in agreement with the observation by others that C2-ceramide failed to induce NF-κB over a range of concentration and duration (12). In our experiments, treatment with ceramide consistently resulted in a reduction of the steady state levels of phosphorylated IκBα which could be observed within 30 min of treatment. At this stage, it is not clear whether this reduction was caused by enhanced degradation of phosphorylated IκBα or by dephosphorylation.

In the presence of ceramide, IκB degradation could be induced by suboptimal doses of OA indicating that ceramide can also potentiate the effects of OA. This observation was unexpected, especially since several ceramide-mediated effects such as apoptosis induced by irradiation and certain chemicals (30), or ceramide- and TNF-induced downregulation of c-myc mRNA (31) are abrogated by OA. These opposing effects have been attributed to the fact that ceramide activates the phosphatase ceramide-activated phosphatase which, itself, is inhibited by OA. Ceramide-induced pathways thus appear to separate into OA-sensitive and -resistant pathways, probably dependent on whether ceramide-activated phosphatase is involved or not.

Stimulation of PKC by PMA in T-cells (32) has been shown to induce IκBα phosphorylation and partial degradation (33). Our findings shown that PMA-induced IκB degradation is enhanced by membrane-permeable ceramide. In LN T-cells, IκBα degradation induced by ceramide and PMA occurred with slower kinetics than for ceramide and OA. In this context, it is noteworthy that DAG, the physiological counterpart of PMA, has been reported to be capable of activating aSMase which, in turn, will result in enhanced ceramide production. Based on these findings, a pathway for TNF-induced NF-κB activation was proposed involving a cooperative relationship between the two lipid second messengers DAG and ceramide (10, 28). As mentioned earlier, however, this notion has been challenged by experiments which show that TNF stimulation can induce NF-κB activation much faster than ceramide generation could be measured (34) and NF-κB activation in the absence of TNF-induced ceramide formation has also been described (12, 13, 29, 35).

As is the case for OA and ceramide, PKC activation and ceramide also appear to exert opposing biological effects. A protective role has been demonstrated for PKC in ceramide-mediated apoptosis induced via a range of stimuli (36–38). The synergistic effect for C2-ceramide and phorbol esters at the level of IκB degradation are not in agreement with the observations by Gamard *et al.* (34), who showed that C2- and C6-ceramide can inhibit the PMA-induced activation of NF-κB in Jurkat T-cells. The reason for this difference is not clear, but could be due to the fact that different experimental approaches were used. In the study of Gamard *et al.*, Jurkat T-cells were pre-treated with ceramide for 45 minutes prior to PMA stimulation, whereas in our experiments with primary LN T-cells and TpM(803) T-cells, ceramide and PMA treatment were added concurrently. That different stimulation regimes can indeed influence responses to PMA, OA or ceramide was recently confirmed by experiments in our lab, in which IκB degradation induced by these three compounds was affected by prior stimulation of the T-cells with either ConA or PMA (P.F. and D.D. unpublished).

The way in which ceramide enhances IκB degradation is not known. Both SMase and ceramide have been shown in *in vitro* studies to activate a serine-specific protease resulting in the degradation of IκB (19). Recently, PKCζ was shown to be involved in the activation of NF-κB through phosphorylation of IκB (39, 40). Since ceramide can activate PKCζ, it is conceivable that ceramide-activated PKCζ contributes to NF-κB activation by feeding into the pathways which is also stimulated upon OA or PMA treatment. Apart from

affecting IκB, PKCζ is also involved in regulating the transcriptional activity of NF-κB that has been released from its inhibitor. This involves the phosphorylation of the RelA subunit of NF-κB through a pathway entailing the activation of PKCζ and p21(ras) (41). Our studies focussed on a single step—IκB degradation—in the NF-κB activation cascade and it will be of interest to see whether these effects are also reflected at the transcriptional level.

In summary, we show that ceramide can potentiate signals involved in IκB degradation, delivered by the phorbol ester PMA or the inhibitor OA, two compounds which regulate PKC- or PP2A-dependent NF-κB activation pathways, respectively. Even though ceramide alone may not function to activate NF-κB directly, the cooperation of ceramide with these pathways supports a role for ceramide in a multistep NF-κB activation process. Our data also emphasise the complex interplay between signalling pathways involving sphingolipids and glycerolipids and underscore the complexity of their regulation.

ACKNOWLEDGMENTS

This work was supported by the Swiss National Science Foundation (Grant Nr 31-43′328.95 to D.D. and Grant 31-44′407.95 to P.F.) and the Swiss Cancer League (KFS 625-2-1998).

REFERENCES

1. Hannun, Y. A., Obeid, L. M., and Dbaibo, G. S. (1996) *Ceramide, A Novel Second Messenger and Lipid Mediator,* Plenum Press, New York, NY.
2. Jayadev, S., and Hannun, Y. A. (1996) *J. Lipid Mediat. Cell Signal* **14,** 295–301.
3. Hannun, Y. A. (1996) *Science* **274,** 1855–1859.
4. Ballou, L. R., Laulederkind, S. J., Rosloniec, E. F., and Raghow, R. (1996) *Biochimica Et Biophysica Acta* **1301,** 273–287.
5. Verma, I. M., Stevenson, J. K., Schwarz, E. M., Van Antwerp, D., and Miyamoto, S. (1995) *Genes Dev.* **9,** 2723–2735.
6. Baldwin, A. S. J. (1996) *Annu. Rev. Immunol.* **14,** 649–683.
7. Mercurio, F., and Manning, A. M. (1999) *Curr. Opin. Cell Biol.* **11,** 226–232.
8. Baichwal, V. R., and Baeuerle, P. A. (1997) *Curr. Biol.* **7,** R94–R96.
9. Karin, M. (1998) *Cancer J. Sci. Am.* **4, Suppl. 1,** S92–S99.
10. Schutze, S., Machleidt, T., and Kronke, M. (1994) *J. Leukoc. Biol.* **56,** 533–541.
11. Schutze, S., Potthoff, K., Machleidt, T., Berkovic, D., Wiegmann, K., and Kronke, M. (1992) *Cell* **71,** 765–776.
12. Dbaibo, G. S., Obeid, L. M., and Hannun, Y. A. (1993) *J. Biol. Chem.* **268,** 17762–17766.
13. Betts, J. C., Agranoff, A. B., Nabel, G. J., and Shayman, J. A. (1994) *J. Biol. Chem.* **269,** 8455–8458.
14. Westwick, J. K., Bielawska, A. E., Dbaibo, G., Hannun, Y. A., and Brenner, D. A. (1995) *J. Biol. Chem.* **270,** 22689–22692.
15. Latinis, K. M., and Koretzky, G. A. (1996) *Blood* **87,** 871–875.
16. Higuchi, M., Singh, S., Jaffrezou, J. P., and Aggarwal, B. B. (1996) *J. Immunol.* **157,** 297–304.
17. Kuno, K., Sukegawa, K., Ishikawa, Y., Orii, T., and Matsushima, K. (1994) *Int. Immunol.* **6,** 1269–1272.
18. Boucher, L. M., Wiegmann, K., Futterer, A., Pfeffer, K., Machleidt, T., Schutze, S., Mak, T. W., and Kronke, M. (1995) *J. Exp. Med.* **181,** 2059–2068.
19. Machleidt, T., Wiegmann, K., Henkel, T., Schutze, S., Baeuerle, P., and Krönke, M. (1994) *J. Biol. Chem.* **269,** 13760–13765.
20. Thevenin, C., Kim, S. J., Rieckmann, P., Fujiki, H., Norcross, M. A., Sporn, M. B., Fauci, A. S., and Kehrl, J. H. (1990) *New Biol.* **2,** 793–800.
21. DiDonato, J. A., Hayakawa, M., Rothwarf, D. M., Zandi, E., and Karin, M. (1997) *Nature* **388,** 548–554.
22. Weiss, A., Imboden, J., Hardy, K., Manger, B., Terhorst, C., and Stobo, J. (1986) *Annu. Rev. Immunol.* **4,** 593–619.
23. Dobbelaere, D. A., Prospero, T. D., Roditi, I. J., Kelke, C., Baumann, I., Eichhorn, M., Williams, R. O., Ahmed, J. S., Baldwin, C. L., Clevers, H., et al., (1990) *Infect. Immun.* **58,** 3847–3855.
24. Mastro, A., and Pepin, K. (1980) *Cancer Res.* **40,** 3307–3312.
25. Dobbelaere, D. A., Coquerelle, T. M., Roditi, I. J., Eichhorn, M., and Williams, R. O. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 4730–4734.
26. Palmer, G. H., Machado, J. J., Fernandez, P., Heussler, V., Perinat, T., and Dobbelaere, D. A. (1997) *Proc. Natl. Acad. Sci. USA* **94,** 12527–12532.
27. Ivanov, V., Stein, B., Baumann, I., Dobbelaere, D. A., Herrlich, P., and Williams, R. O. (1989) *Mol. Cell Biol.* **9,** 4677–4686.
28. Schutze, S., Wiegmann, K., Machleidt, T., and Kronke, M. (1995) *Immunobiology* **193,** 193–203.
29. Johns, L. D., Sarr, T., and Ranges, G. E. (1994) *J. Immunol.* **152,** 5877–5882.
30. Song, Q., Baxter, G. D., Kovacs, E. M., Findik, D., and Lavin, M. F. (1992) *J. Cell Physiol.* **153,** 550–556.
31. Wolff, R. A., Dobrowsky, R. T., Bielawska, A., Obeid, L. M., and Hannun, Y. A. (1994) *J. Biol. Chem.* **269,** 19605–19609.
32. Traenckner, E. B., Pahl, H. L., Henkel, T., Schmidt, K. N., Wilk, S., and Baeuerle, P. A. (1995) *Embo Journal* **14,** 2876–2883.
33. Steffan, N. M., Bren, G. D., Frantz, B., Tocci, M. J., O Neill, E. A., and Paya, C. V. (1995) *J. Immunol.* **155,** 4685–4691.
34. Gamard, C. J., Dbaibo, G. S., Liu, B., Obeid, L. M., and Hannun, Y. A. (1997) *J. Biol. Chem.* **272,** 16474–16481.
35. Jayadev, S., Hayter, H. L., Andrieu, N., Gamard, C. J., Liu, B., Balu, R., Hayakawa, M., Ito, F., and Hannun, Y. A. (1997) *J. Biol. Chem.* **272,** 17196–17203.
36. Obeid, L. M., Linardic, C. M., Karolak, L. A., and Hannun, Y. A. (1993) *Science* **259,** 1769–1771.
37. Jarvis, W. D., Fornari, F. A., Jr., Browning, J. L., Gewirtz, D. A., Kolesnick, R. N., and Grant, S. (1994) *J. Biol. Chem.* **269,** 31685–31692.
38. Haimovitz-Friedman, A., Kan, C. C., Ehleiter, D., Persaud, R. S., McLoughlin, M., Fuks, Z., and Kolesnick, R. N. (1994) *J. Exp. Med.* **180,** 525–535.
39. Lallena, M. J., Diaz-Meco, M. T., Bren, G., Paya, C. V., and Moscat, J. (1999) *Mol. Cell. Biol.* **19,** 2180–2188.
40. Diaz Meco, M. T., Dominguez, I., Sanz, L., Dent, P., Lozano, J., Municio, M. M., Berra, E., Hay, R. T., Sturgill, T. W., and Moscat, J. (1994) *Embo J.* **13,** 2842–2848.
41. Anrather, J., Csizmadia, V., Soares, M. P., and Winkler, H. (1999) *J. Biol. Chem.* **274,** 13594–13603.