Ceramide generation by two distinct pathways in tumor necrosis factor α-induced cell death

Ghassan S. Dbaibo[a,b,*], Wissal El-Assaad[a,b], Armand Krikorian[b], Bin Liu[d], Karim Diab[b], Nadine Z. Idriss[b], Marwan El-Sabban[c], Timothy A. Driscoll[e], David K. Perry[f], Yusuf A. Hannun[f]

[a] *Department of Pediatrics, American University of Beirut, Beirut, Lebanon*
[b] *Department of Biochemistry, American University of Beirut, Beirut, Lebanon*
[c] *Department of Human Morphology, American University of Beirut, Beirut, Lebanon*
[d] *Neuropharmacology Section, Laboratory of Pharmacology and Chemistry, National Institute for Environmental Health Sciences, Research Triangle Park, NC 27709, USA*
[e] *Department of Pediatrics, Duke University, Durham, NC 27710, USA*
[f] *Department of Biochemistry and Molecular Biology, Medical College of South Carolina, Charleston, SC 29425, USA*

Received 20 May 2001; revised 16 June 2001; accepted 18 June 2001

First published online 23 July 2001

Edited by Guido Tettamanti

Abstract Ceramide accumulation in the cell can occur from either hydrolysis of sphingomyelin or by de novo synthesis. In this study, we found that blocking de novo ceramide synthesis significantly inhibits ceramide accumulation and subsequent cell death in response to tumor necrosis factor α. When cells were pre-treated with glutathione, a proposed cellular regulator of neutral sphingomyelinase, inhibition of ceramide accumulation at early time points was achieved with attenuation of cell death. Inhibition of both pathways achieved near-complete inhibition of ceramide accumulation and cell death indicating that both pathways of ceramide generation are stimulated. This illustrates the complexity of ceramide generation in cytokine action. © 2001 Published by Elsevier Science B.V. on behalf of the Federation of European Biochemical Societies.

Key words: Cell death; Apoptosis; Ceramide; Sphingomyelinase; Ceramide synthase

1. Introduction

Ceramide has been proposed as a coordinator of the response to stress including the propagation of the apoptotic signal produced not only by death receptors, but also by sensors of genotoxic stress such as p53 [1,2]. Generation of ceramide from the hydrolysis of membrane sphingomyelin by the action of sphingomyelinases is a primary mechanism for rapidly increasing ceramide levels in the cell. Several sphingomyelinases have been identified, but not all of them have been fully characterized [3]. The neutral magnesium-dependent sphingomyelinase (N-SMase) has been linked to apoptosis mediated by tumor necrosis factor α (TNFα), Fas, serum starvation, and some chemotherapeutic agents [4,5]. Recently, glutathione (GSH) was shown to inhibit the activation of N-SMase both in partially purified preparations and in cells treated with TNFα [5,6]. GSH is the most abundant thiol-containing cellular antioxidant and it is rapidly depleted in response to various apoptotic stimuli, especially those resulting in the generation of reactive oxygen intermediates (ROI) [7,8]. This, along with its demonstrated effects on N-SMase, raised the possibility that N-SMase may be specifically coupled to the oxidative stress response via GSH. Increased acidic sphingomyelinase (A-SMase) activity has also been shown in response to γ-irradiation, Fas, and TNFα [9–11]. The role of other sphingomyelinases has not yet been determined.

Ceramide can also be generated by de novo synthesis via condensation of L-serine and palmitoyl-CoA followed by reduction of the resulting ketosphinganine to sphinganine. *N*-acylation of sphinganine by ceramide synthase produces dihydroceramide, which is then desaturated between carbons four and five to give ceramide. In addition, cellular sphingosine can be *N*-acylated by ceramide synthase to give ceramide. De novo synthesis of ceramide has been implicated in ceramide accumulation in response to retinoic acid [12], and activation of ceramide synthase has been suggested as the mechanism for the elevation of ceramide and induction of apoptosis in response to the chemotherapeutic agent daunorubicin [13].

The role of ceramide in apoptosis, especially that induced by TNFα, has been the subject of some controversy [14,15]. In this study, we show that TNFα treatment of either MCF7 breast carcinoma or L929 murine fibrosarcoma cells results in the activation of two pathways of ceramide generation: one that is inhibitable by GSH (and is probably mediated by N-SMase), and the second is dependent on de novo synthesis of ceramide and is inhibitable by fumonisin B1 (FB1). We show that by blocking both pathways, significant delay of cell death is achieved, suggesting an important role for the accumulated ceramide in the propagation of TNFα-induced apoptosis.

*Corresponding author. Department of Pediatrics, American University of Beirut-Medical Center, P.O. Box 113/6044, Beirut, Lebanon. Fax: (961)-1-370781.
E-mail address: gdbaibo@aub.edu.lb (G.S. Dbaibo).

Abbreviations: TNFα, tumor necrosis factor α; N-SMase, neutral sphingomyelinase; GSH, glutathione; FB1, fumonisin B1; ROI, reactive oxygen intermediate

PII: S0014-5793(01)02625-4

2. Materials and methods

2.1. Cell culture and viability assays

The TNFα-sensitive MCF7 (a kind gift from Dr. Vishva Dixit, Genentech, south San Francisco, CA, USA) and L929 (American Type Culture Collection, Rockville, USA) cell lines were grown in RPMI1640 and Dulbecco's modified Eagle's medium, respectively. Cell viability was determined either by the ability to exclude trypan blue or the ability to cleave the tetrazolium salt WST-1 to formazan by mitochondrial dehydrogenases in viable cells and by following the manufacturer's procedure (Boehringer Mannheim, Germany). MCF7 cells were treated with TNFα at 1 nM, whereas L929 cells were treated with TNFα at 0.1 nM, unless indicated otherwise. Cells were treated with GSH at 10 mM unless indicated otherwise.

Apoptotic cells were identified as follows: L929 cells were seeded at 5×10^4 cells on 10-mm cover slips and rested overnight. Following treatment, the cells were then stained in a (0.5 μg/ml) solution Hoechst 33342 (Molecular Probes) for 10 min, then fixed with 4% formaldehyde. The stained nuclei were visualized by fluorescence microscopy using a FITC range filter on a Zeiss confocal microscope fitted with a Nikon 6006 camera. Apoptotic cells were defined by the condensation of nuclear chromatin, its fragmentation, or its margination to the nuclear membrane. The percentage of apoptotic cells was determined after counting at least 200 cells. Experiments were done in duplicate at least twice.

2.2,. Ceramide measurement

Cells were seeded at 1×10^6 cells/ml in a 100-mm Petri dish in 5 ml volume, rested overnight, then treated with TNFα. Lipids were collected as described [16]. Ceramide was measured using a modified diacylglycerol kinase assay using external ceramide standards as described previously, and expressed as a ratio of ceramide over lipid phosphate [17].

For assessing de novo ceramide synthesis, labeling with palmitate was utilized. MCF7 cells were seeded at 5×10^5 cells/well in 6-well culture plates in RPMI with 10% fetal bovine serum (3.5 ml of medium/well). Cells were allowed to grow for 24 h and were then treated with 3 μCi/well of [9,10-^{3}H(N)]palmitate (New England Nuclear) and immediately treated with vehicle control, TNFα or FB1. Lipids were extracted from harvested cells and palmitate incorporation into newly synthesized ceramide was measured as described [18]. Results are expressed as cpm of ceramide/nmol lipid-Pi and are the mean ± the standard deviation of triplicate samples.

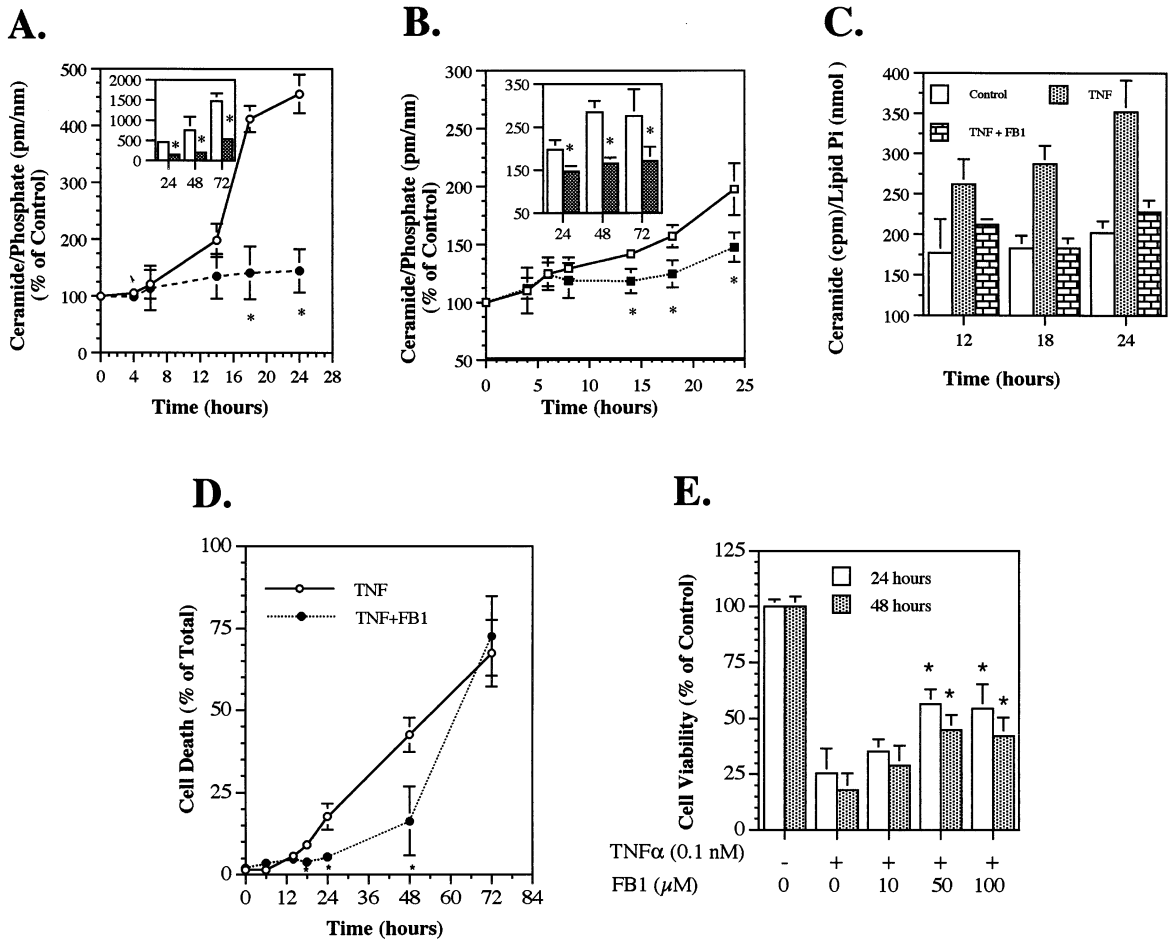

Fig. 1. Inhibition of TNFα-induced ceramide accumulation and cell death by FB1. A: Inhibition of TNFα-induced ceramide accumulation in MCF7 cells pre-treated with FB1. MCF7 cells were treated in duplicate with TNFα with (closed circles and bars) or without (open circles and bars) FB1 (100 μM). Ceramide levels were normalized to lipid phosphate and are presented as percentage of similarly treated cells, but in the absence of TNFα treatment. B: Inhibition of TNFα-induced ceramide accumulation in L929 cells pre-treated with FB1. L929 cells were seeded and treated as in (A). TNFα (open squares and bars), TNFα and FB1 (closed squares and bars). C: De novo ceramide synthesis by TNFα and its inhibition by FB1 in MCF7 cells. MCF7 cells were labeled with [^{3}H]palmitate as described in Section 2. Cells were treated with vehicle (clear bar), TNFα (stippled bar), or the combination of TNFα and FB1 50 μM (brick bar). D: Inhibition of TNFα-induced cell death in MCF7 cells pre-treated with FB1. MCF7 cells were treated as in (A) and trypan blue-positive cells were counted and are presented as a percent of total cells counted. E: Inhibition of TNFα-induced cell death in L929 cells pre-treated with FB1. L929 cells were seeded at a density of 5×10^3 cells/well of a 96-well plate rested for 24 h and treated in triplicate with vehicle, TNFα, with or without the indicated concentrations of FB1. Cleavage of WST1 is presented as percent of vehicle treated cells. Data represent at least three experiments and bars indicate S.E.M. *Represents statistically significant differences from corresponding TNFα-treated control.

2.3. GSH measurement

Cells were seeded at 1×10^6 cells/ml in a 100-mm Petri dish, rested overnight, and then treated accordingly. At the desired time points, cells were harvested by trypsinization, washed twice with ice-cold phosphate-buffered saline, then lysed with 150 μl of cold water. Proteins were precipitated with 2% 5-sulfosalicylic acid and were separated from the supernatant by centrifugation. GSH content in the supernatant was measured using the Griffith modification of Tietze's enzymatic procedure as described [6].

2.4. Sphingomyelinase activity assay

N-SMase was partially purified as described previously [5]. Activity of sphingomyelinases was assayed by a mixed micelle assay as described [6] using [^{14}C]sphingomyelin as a substrate.

2.5. Statistical analysis

Statistically significant differences were determined by using one-way analysis of variance followed by Bonferroni's post-hoc test. A P value of less than 0.05 was considered significant. Unless otherwise indicated, data is represented as mean ± S.E.M. based on at least three independent experiments.

3. Results

3.1. TNFα induces de novo ceramide synthesis

Our previous studies suggested that N-SMase contributed only part of the ceramide response to TNFα. We therefore decided to investigate the role of de novo ceramide synthesis in TNFα-induced ceramide accumulation and cell death. We utilized two cell lines that have been used extensively in exploring the death-inducing effects of TNFα: the MCF7 breast carcinoma epithelial cell line and the L929 murine fibrosarcoma cell line. In both cell lines, treatment with TNFα results in gradual accumulation of cellular ceramide followed by cell death (Fig. 1). We utilized the mycotoxin FB1 which is produced by some strains of *Fusarium moniliforme*. FB1 is a structural sphingolipid analog which blocks the acylation step in ceramide synthesis carried by ceramide synthase by competing with sphinganine, sphingosine, and possibly also the fatty acyl CoA [19]. In vitro, FB1 inhibits ceramide synthase with an apparent K_i of 0.05–0.1 μM, but higher concentrations (10–30 μM) are required for inhibition of de novo synthesis in cells. FB1 significantly inhibited the accumulation of ceramide following TNFα over control cells treated with FB1 alone (Fig. 1A,B). Notably, the degree of inhibition of ceramide accumulation in the L929 cell line was comparatively less, indicating that de novo ceramide synthesis may contribute a smaller fraction of the total ceramide in this cell line. De novo ceramide synthesis in response to TNFα was verified by showing [^{3}H]palmitate incorporation in newly synthesized ceramide following TNFα treatment of MCF7 cells and its inhibition by FB1 (Fig. 1C).

We next evaluated the effects of FB1 on TNFα-induced cell death. Cells were treated with TNFα in the presence or absence of FB1 and then evaluated by an inverted microscope at 14 and 24 h. In the presence of FB1, 80% of MCF7 cells and 50% of L929 cells remained attached to the plate and exhibited normal morphology and spread, whereas in the absence of FB1 more than 90% of cells had detached by 24 h in both cell lines. When dead cells were quantified by trypan blue uptake, it was found that FB1 provided significant protection from the cytotoxic effects of TNFα lasting until 48 h following treatment (Fig. 1D). By 72 h, little protection from cell death was evident in MCF7 cells treated with FB1. Similar, but less dramatic results were observed in L929 cells (Fig. 1E). Thus, in the TNFα-stimulated pathway of cell death, de novo ceramide synthesis appears to be necessary for at least part of ceramide accumulation and subsequent cell death.

Previously, it was suggested that TNFα-induced ceramide accumulation and subsequent cell death were mediated by the activation of N-SMase. Because of these considerations, we evaluated the effects of FB1 on the activity of partially purified N-SMase. Incubation of N-SMase with a range of concentrations of FB1 from 0.1 nM to 100 μM did not affect its activity (data not shown). In other studies, FB1 did not affect the activity of A-SMase either (data not shown).

TNFα treatment of MCF7 cells results in the loss of cellular GSH, which releases the inhibition of N-SMase and leads to generation of ceramide from sphingomyelin hydrolysis [5]. Therefore, we examined whether FB1 indirectly affects N-SMase activity by modulating cellular GSH levels. We

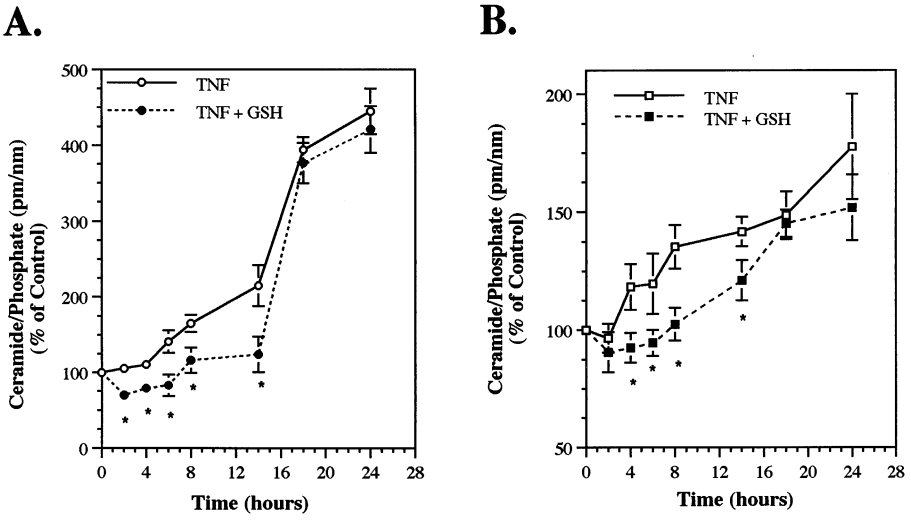

Fig. 2. Inhibition of TNFα-induced ceramide accumulation and cell death by GSH. A,B: Inhibition of the early TNFα-induced ceramide response in MCF7 or L929 cells by GSH. MCF7 (A) or L929 (B) cells were prepared as in Fig. 1A and pre-treated for 1 h with GSH prior to treatment with TNFα. Data represent at least three experiments and bars indicate S.E.M. *Represents statistically significant differences from corresponding TNFα treated control in A,B.

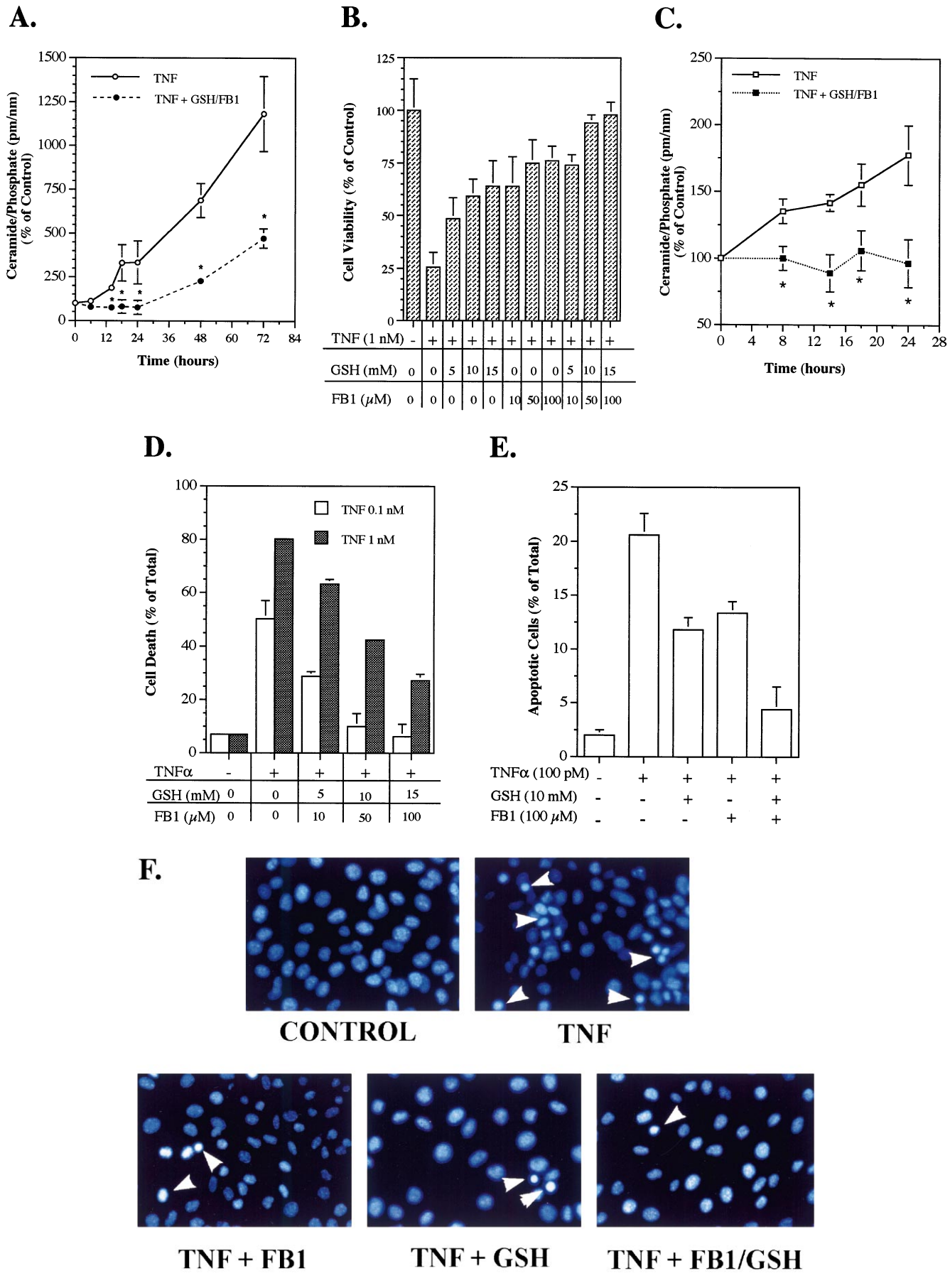
A.
Ceramide/Phosphate (pm/nm) (% of Control)
TNF
TNF + GSH/FB1
Time (hours)
B.
Cell Viability (% of Control)
TNF (1 nM)
GSH (mM)
FB1 (μM)
C.
Ceramide/Phosphate (pm/nm) (% of Control)
TNF
TNF + GSH/FB1
Time (hours)
D.
Cell Death (% of Total)
TNF 0.1 nM
TNF 1 nM
TNFα
GSH (mM)
FB1 (μM)
E.
Apoptotic Cells (% of Total)
TNFα (100 pM)
GSH (10 mM)
FB1 (100 μM)
F.
CONTROL
TNF
TNF + FB1
TNF + GSH
TNF + FB1/GSH

Fig. 3. Inhibition of TNFα-induced ceramide accumulation and cell death by combined use of GSH and FB1. A: Inhibition of ceramide accumulation in MCF7 cells. Cells were prepared as in Fig. 1A and pre-treated with GSH and FB1 (100 μM) for 1 h before treatment with TNFα. B: Inhibition of cell death in MCF7 cells. MCF7 cells were seeded at 5×10^3 cells/well in a 96-well plate in triplicates and treated with the indicated concentrations of GSH, FB1, or TNFα. Viability was measured by WST1 cleavage at 24 h. A representative experiment is shown. C: Inhibition of ceramide accumulation in L929 cells (as in Fig. 3A). D: Inhibition of cell death in L929 cells in response to TNFα. L929 cells were seeded at 5×10^4 cells/well in a 24-well plate and rested overnight prior to treatment with the indicated concentrations of TNFα, GSH, and FB1. Viability at 24 h was assessed by trypan blue uptake. E: Inhibition of TNFα-induced apoptosis in L929 cells. L929 cells were seeded and treated with TNFα with or without GSH or FB1 as indicated for 24 h. Apoptotic cells were counted after staining with Hoechst dye and are represented as a percentage of total cells. Floating cells were not included as they were removed by washing. F: Apoptosis in response to various treatments. L929 cells were treated as in (E) and representative fields were photographed. Arrowheads indicate apoptotic cells.
←

found that treatment of MCF7 cells with TNFα caused a drop in GSH levels, as normalized to cellular protein, to 55%, 38%, and 21% of control at 1, 14, and 18 h, respectively. Pre-treatment of MCF7 cells with FB1 did not interfere with the drop of GSH induced by TNFα (data not shown). Similar results were observed in L929 cells where pre-treatment of cells with GSH attenuated the GSH drop from 19% of control to 58%, whereas pre-treatment with FB1 had no effect. These results suggest that FB1 does not interfere with the N-SMase pathway of ceramide generation either directly or indirectly, and that the FB1 inhibition of de novo synthesis of ceramide is independent of regulation of sphingomyelinases.

In order to determine that the effects of FB1 were due to inhibition of ceramide generation, we treated cells with cell-permeable synthetic C_2-ceramide (*N*-acetyl sphingosine) in the presence of TNFα and FB1. We found that at 24 h, TNFα caused 27% cell death which was reduced to 11% by FB1. C_2-ceramide at concentrations of 10, 20, and 40 μM could overcome the inhibition of FB1 and induce cell death at 20, 39, and 78%, respectively. This suggested that the effects of FB1 were specific to the interruption of the ceramide signal.

3.2. GSH regulates ceramide accumulation at the early time points

Next, we examined the relationship of inhibition of de novo synthesis to the inhibition of the GSH-regulated N-SMase pathway. First, the effects of modulation of GSH on TNFα-induced ceramide accumulation were examined in both cell lines. GSH pre-treatment resulted in inhibition of the early accumulation of ceramide in both cell lines that occurred up until 14 h, but it failed to inhibit the late accumulation of ceramide (Fig. 2A,B). As compared to untreated control, cell viability assessed by cleavage of WST-1 at 24 h improved in MCF7 cells from 31% after treatment with TNFα alone to 70% with GSH pre-treatment and 52% with FB1 pre-treatment. In L929 cells, the GSH-induced improvement in cell viability was more pronounced (TNFα 18%, TNFα and GSH 85%, TNFα and FB1 46%). These results confirm and extend previous findings that GSH depletion may contribute to ceramide accumulation and subsequent cell death in response to TNFα. However, it appears that the extent of this contribution may vary between cell lines.

3.3. Independent pathways of ceramide accumulation

These findings led us to suspect the presence of two independent pathways of ceramide generation in response to TNFα treatment in the two cell lines examined. We examined whether it was possible to completely block ceramide generation by blocking both pathways and whether this would provide better protection from the cytotoxic effects of TNFα. Indeed, the combination of FB1 and GSH pre-treatment of MCF7 cells almost completely abolished ceramide accumulation at both early and late time points up to 24 h following treatment (Fig. 3A). Subsequently, ceramide levels started to increase as the effects of the inhibitors were lost. This combination of inhibitors delayed cell death more effectively than either GSH or FB1 alone (Fig. 3B). At later time points, the rising levels of ceramide correlated with a decrease in cell viability (data not shown). Similar results were obtained in L929 cells (Fig. 3C,D). Moreover, the number of L929 cells dying by apoptosis as determined by morphologic criteria in response to TNFα was lower when cells were co-treated with GSH, FB1 or the combination of GSH and FB1 (Fig. 3E,F). Therefore, although the individual contribution of either pathway of ceramide generation may vary with cell type, they appear to play a complementary role. The simultaneous inhibition of both pathways provides a similar degree of inhibition of ceramide accumulation and cell death when both cell lines are compared.

4. Discussion

Our findings suggest that, in response to TNFα treatment, at least two independent pathways of ceramide generation become activated. The GSH-dependent pathway appears to be responsible for the earlier ceramide response and probably involves activation of N-SMase. Our results also suggest that other functions of GSH, such as its antioxidant properties, may contribute to its death-inhibiting activity as evidenced by the discrepancy between its ability to inhibit TNFα-induced death and ceramide accumulation (Fig. 2). More definitive proof of the role of N-SMase requires additional specific pharmacological inhibitors of the enzyme that currently do not exist.

The second pathway involves ceramide synthase, which from this study appears to be responsible for the slow but sustained generation of ceramide occurring several hours following receptor ligation. Both pathways appear to contribute independently to the induction of cell death since inhibition of either one will partially improve cell viability. Moreover, the contribution of the two pathways to the accumulated ceramide pool may differ depending on the cell type involved. This is evident by the different, but complementary effects of FB1 and GSH in the two cell lines used in this study.

Several effector molecules in TNFα-induced apoptosis have been described, and their relationship to ceramide has been examined. Receptor-activated caspases appear to be at the apex of a signaling cascade that results in downstream events that include the accumulation of ceramide. Inhibition of these apical caspases by specific peptide inhibitors, expression of the viral protein CrmA, or overexpression of Bcl-x_L prevents ceramide accumulation (both early and late) and apoptosis

[17,20]. Ceramide has been shown to activate the downstream caspases responsible for cleavage of 'death' substrates and these effects can be blocked by overexpression of Bcl-2. Importantly, Bcl-2 blocks ceramide-induced activation of caspases and cell death without interfering with ceramide accumulation in response to TNFα or other stimuli [17,20–22]. Additionally, a role for ROI in the signaling of TNFα-induced cell death has been proposed, but remains controversial [7,23]. Ceramide has been shown to either induce the formation of ROI or to be generated in response to treatments that specifically increase the production of ROI [24,25]. Interestingly, GSH is the major intracellular antioxidant responsible for reducing ROI. Its added role in regulating N-SMase raises the possibility that it functionally regulates these two pathways.

Our findings are compatible with those of a recent study [26] that showed partial inhibition of cell death and ceramide accumulation by FB1 in bovine cerebral endothelial cells stimulated by a combination of TNFα and cycloheximide. These studies underscore the importance of ceramide accumulation in propagating the cell death signal in response to TNFα and specifically highlight the role for de novo ceramide synthesis in this process. Further studies are needed to determine the roles of the different enzymes involved in ceramide accumulation and their regulation as they may provide key targets for specific regulation of cell death.

Acknowledgements: This work was funded by a grant from the Medical Practice Plan at the American University of Beirut (G.D.) and NIH Grant GM 43825 (Y.H.). Part of this work was done using the equipment and resources from the Research Core Facility Laboratories at the American University of Beirut.

References

[1] Hannun, Y.A. (1996) Science 274, 1855–1859.
[2] Riboni, L., Viani, P., Bassi, R., Prinetti, A. and Tettamanti, G. (1997) Prog. Lipid Res. 36, 153–195.
[3] Liu, B., Obeid, L.M. and Hannun, Y.A. (1997) Semin. Cell Dev. Biol. 8, 311–322.
[4] Mansat, V., Bettaïeb, A., Levade, T., Laurent, G. and Jaffrézou, J.P. (1997) FASEB J. 11, 695–702.
[5] Liu, B., Andrieu-Abadie, N., Levade, T., Zhang, P., Obeid, L.M. and Hannun, Y.A. (1998) J. Biol. Chem. 273, 11313–11320.
[6] Liu, B. and Hannun, Y.A. (1997) J. Biol. Chem. 272, 16281–16287.
[7] Goossens, V., Grooten, J., De Vos, K. and Fiers, W. (1995) Proc. Natl. Acad. Sci. USA 92, 8115–8119.
[8] Van Den Dobbelsteen, D.J., Novel, C.S.I., Schlegel, J., Cotgreave, I.A., Orrenius, S. and Slater, A.F.G. (1996) J. Biol. Chem. 271, 15420–15427.
[9] Cifone, M.G., De Maria, R., Roncaioli, P., Rippo, M.R., Azuma, M., Lanier, L.L., Santoni, A. and Testi, R. (1994) J. Exp. Med. 180, 1547–1552.
[10] Santana, P., Peña, L.A., Haimovitz-Friedman, A., Martin, S., Green, D., McLoughlin, M., Cordon-Cardo, C., Schuchman, E.H., Fuks, Z. and Kolesnick, R. (1996) Cell 86, 189–199.
[11] Boesen-de Cock, J.G.R., Tepper, A.D., de Vries, E., van Blitterswijk, W.J. and Borst, J. (1998) J. Biol. Chem. 273, 7560–7565.
[12] Kalén, A., Borchardt, R.A. and Bell, R.M. (1992) Biochim. Biophys. Acta Lipids Lipid Metab. 1125, 90–96.
[13] Bose, R., Verheij, M., Haimovitz-Friedman, A., Scotto, K., Fuks, Z. and Kolesnick, R.N. (1996) Cell 82, 405–414.
[14] Hofmann, K. and Dixit, V.M. (1998) Trends Biochem. Sci. 23, 374–377.
[15] Kolesnick, R. and Hannun, Y.A. (1999) Trends Biochem. Sci. 24, 224–225.
[16] Bligh, E.G. and Dyer, W.J. (1959) Can. J. Biochem. Phys. 37, 911–917.
[17] Dbaibo, G.S., Perry, D.K., Gamard, C.J., Platt, R., Poirier, G.G., Obeid, L.M. and Hannun, Y.A. (1997) J. Exp. Med. 185, 481–490.
[18] Perry, D.K., Carton, J., Shah, A.K., Meredith, F., Uhlinger, D.J. and Hannun, Y.A. (2000) J. Biol. Chem. 275, 9078–9084.
[19] Merrill Jr., A.H., Van Echten, G., Wang, E. and Sandhoff, K. (1993) J. Biol. Chem. 268, 27299–27306.
[20] El-Assaad, W., El-Sabban, M., Awaraji, C., Abboushi, N. and Dbaibo, G.S. (1998) Biochem. J. 336, 735–741.
[21] Zhang, J., Alter, N., Reed, J.C., Borner, C., Obeid, L.M. and Hannun, Y.A. (1996) Proc. Natl. Acad. Sci. USA 93, 5325–5328.
[22] Allouche, M., Bettaieb, A., Vindis, C., Rousse, A., Grignon, C. and Laurent, G. (1997) Oncogene 14, 1837–1845.
[23] Jacobson, M.D. (1996) Trends Biochem. Sci. 21, 83–86.
[24] García-Ruiz, C., Colell, A., Marí, M., Morales, A. and Fernández-Checa, J.C. (1997) J. Biol. Chem. 272, 11369–11377.
[25] Quillet-Mary, A., Jaffrézou, J.P., Mansat, V., Bordier, C., Naval, J. and Laurent, G. (1997) J. Biol. Chem. 272, 21388–21395.
[26] Xu, J., Yeh, C.H., Chen, S., He, L., Sensi, S.L., Canzoniero, L.M.T., Choi, D.W. and Hsu, C.Y. (1998) J. Biol. Chem. 273, 16521–16526.