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Sphingolipid signalling: molecular basis and role in TNF-α-induced cell death

Sophie Malagarie-Cazenave, Nathalie Andrieu-Abadie, Bruno Ségui, Valérie Gouazé, Claudine Tardy, Olivier Cuvillier and Thierry Levade

Various lipidic molecules serve as second messengers for transducing signals from the cell surface to the cell interior and trigger specific cellular responses. Sphingolipids represent a complex group of lipids that have recently emerged as new transducers in eukaryotic cells. Several sphingolipid molecules are able to modulate cell growth, differentiation and death. This review summarises current knowledge of the signalling functions of sphingolipids, especially in the regulation of tumour necrosis factor α (TNF-α)-mediated cytotoxic effects. TNF-α is a multifaceted cytokine that controls a wide range of immune responses in mammals, including induction of programmed cell death (also called apoptosis). On the basis of recent observations, a working model is proposed for the molecular mechanisms underlying regulation of sphingolipid generation following TNF-α receptor 1 activation. The implications of these findings for the development of future pharmacological strategies to prevent the cytotoxic TNF-α response and subsequent cellular dysfunctions (as seen in various human diseases) are discussed.

All the authors are at INSERM U.466, Laboratoire de Biochimie, CHU Rangueil, 1 avenue Jean Poulhès, TSA 50032, 31059 Toulouse cedex 9, France. Tel: +33 561 32 20 60; Fax: +33 561 32 20 84.

Sophie Malagarie-Cazenave, PhD student. E-mail: sophie.malagarie@toulouse.inserm.fr
Nathalie Andrieu-Abadie, INSERM Investigator. E-mail: nandrieu@toulouse.inserm.fr
Bruno Ségui, Assistant Professor. E-mail: seguib@yahoo.fr
Valérie Gouazé, Postdoctoral Researcher. E-mail: GouazeV@jwci.org
Claudine Tardy, PhD student. E-mail: claudine.tardy@toulouse.inserm.fr
Olivier Cuvillier, INSERM Investigator. E-mail: olivier.cuvillier@toulouse.inserm.fr
Thierry Levade (corresponding author), Professor of Biochemistry and Molecular Biology. E-mail: levade@toulouse.inserm.fr

Laboratory website: http://www.toulouse.inserm.fr/srv/bmip/fdrech/unites/U466/levade.html

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Mammalian cell populations are continually controlled through a delicate equilibrium between cell division and cell death. Apoptosis is a sophisticated programme that allows maintenance of this balance. Alterations in this programme or its regulatory mechanisms probably result in either degenerative or malignant diseases. Apoptotic cell death is achieved through activation of complex pathways involving specific cysteinyl proteases – the caspases – which can be triggered by signals emanating from the plasma membrane or the cell interior (reviewed in Ref. 1). Recently, several sphingolipids have emerged as cellular constituents that are able to promote, mediate or counterbalance apoptosis (Refs 2, 3). This review discusses the contribution of this particular class of lipids to the cell death programme initiated by the binding of the pleiotropic cytokine tumour necrosis factor α (TNF-α) to its cell-surface receptors.

TNF-α: effects and signalling pathways

TNF-α, previously termed cachectin because of its wasting effect, is the prototypic member of a superfamily of cytokines that include Fas (CD95/APO-1) ligand (FasL) and CD40L (Refs 4, 5, 6) and that interact with a large number of related receptors. Activation of these receptors by ligand binding leads to many diverse activities, such as cell proliferation, differentiation and apoptosis. TNF-α is mainly produced by monocytes and macrophages, and is viewed as a primary mediator of immune regulation, septic shock and the inflammatory response, as well as an anti-tumour factor (Ref. 4).

The wide range of TNF-α activities is explained by the presence of two types of TNF receptors (TNFRs) on the cell surface of almost all mammalian cell types: p55 TNFR (also known as TNFR1, CD120a or TNFRSF1A) and p75 TNFR (TNFR2, CD120b or TNFRSF1B) (for the standardised nomenclature and synonyms, see http://www.gene.ucl.ac.uk/nomenclature/genefamily/tnftop.html). These receptors are devoid of any intrinsic kinase activity. They belong to a particular family of about 30 identified members, most of of which are type 1 membrane proteins (with an extracellular N-terminus and an intracellular C-terminus) that display high sequence homologies and possess similar biological functions, including regulation of cell growth and death (Ref. 7). In particular, several members of this family, known as 'death receptors', contain a homologous sequence in their C-terminal intracytoplasmic domain called the 'death domain', which is essential for apoptosis signalling. Most of the biological effects of TNF-α appear to be mediated by TNFR1 (Ref. 5).

Signalling pathways initiated by TNF-α are not fully understood. Trimerisation of TNFR1, initiated by ligand binding, activates a complex signalling network that transduces the actions of TNF-α (Fig. 1). These pathways involve protein phosphorylation and dephosphorylation events, activation of several phospholipases (including sphingomyelinases, see below) and transcription

Figure 1. Signalling pathways regulated by TNF-α–TNFR1 (legend). *See next page for figure.* Only the most widely accepted pathways implicated in apoptosis are presented here; not all adaptors, transducers or enzymes are indicated (for more details see Refs 6, 8 and 52, and the recommended further resources). Upon binding of tumour necrosis factor α (TNF-α), trimerisation of the TNF receptor TNFR1 induces recruitment of several proteins to the cytoplasmic 'death domain' (DD) of the receptor. On the one hand, association of RIP and TRAF2 to TRADD elicits the activation of two major transcription factors: nuclear factor NF-κB and activator protein AP-1. When retained in the cytoplasm by the IκB inhibitory protein, the NF-κB dimer is inactive; in response to TNF-α, IκB becomes phosphorylated and degraded, allowing NF-κB to translocate to the nucleus. AP-1-mediated gene induction results from activation of JNK via TRAF2. The transcriptional activity of NF-κB and AP-1 results in suppression of apoptosis. On the other hand, recruitment of FADD to TRADD allows activation of initiator caspases (e.g. caspase-8). The active heterodimeric caspase-8 then activates executioner caspases (e.g. caspase-3) (not shown). Caspase-8 also cleaves the Bcl-2 family member Bid, the truncated form of which (tBid) translocates to mitochondria and activates the release of apoptogenic mitochondrial factors such as cytochrome *c*. Together with ATP and apoptotic protease activating factor 1 (APAF1), cytochrome *c* promotes the activation of caspase-9 (not shown), which in turn activates executioner caspases. The caspase cascade activated downstream of mitochondria can be inhibited by the Bcl-2 or Bcl-x_L proteins. FADD, Fas-associated death domain protein; FAN, factor associated with neutral sphingomyelinase activation; JNK, c-Jun N-terminal kinase; RIP, receptor-interacting protein; TRADD, TNFR-associated death domain protein; TRAF2, TNFR-associated factor 2 **(fig001tlt)**.

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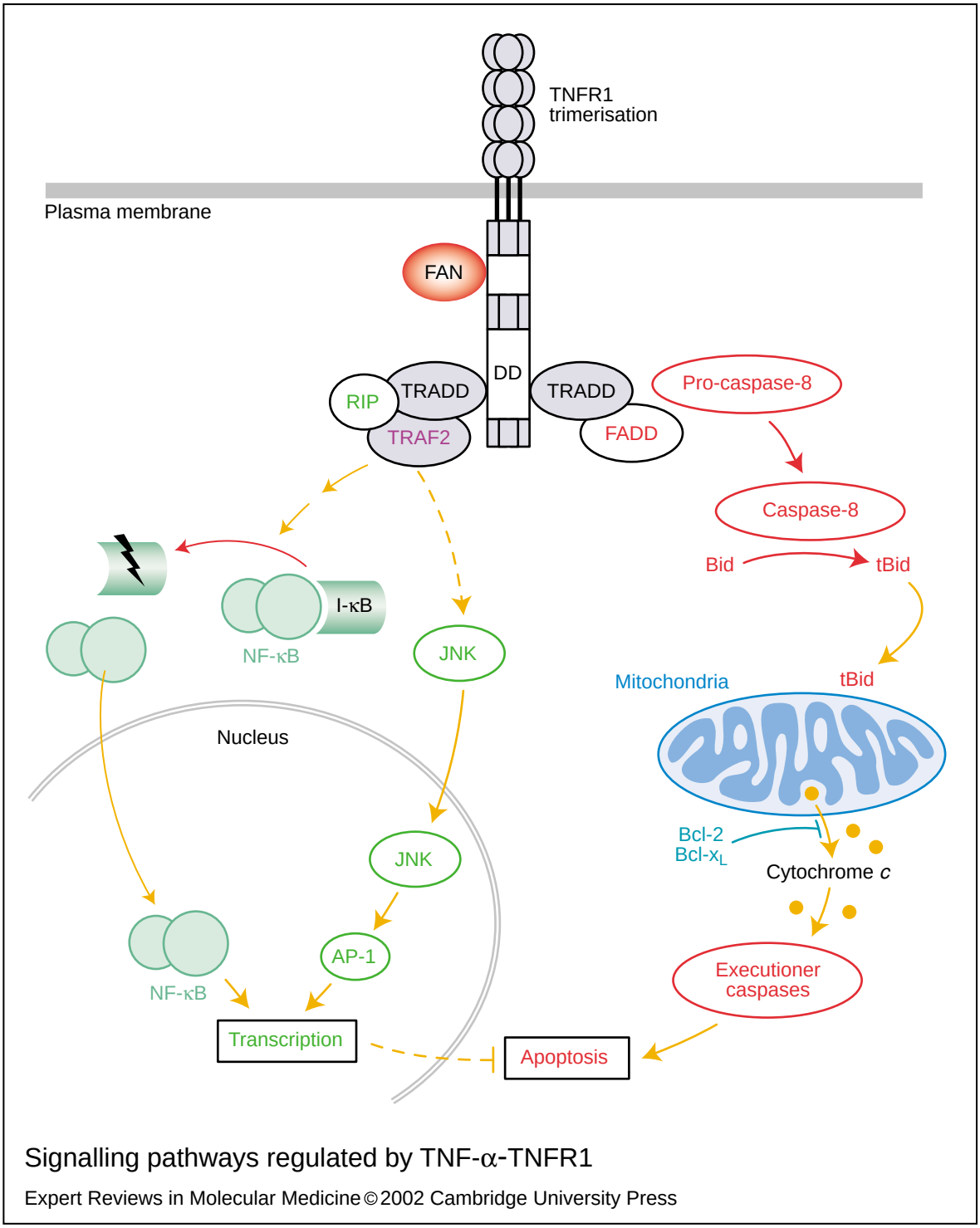

Figure 1. Signalling pathways regulated by TNF-α–TNFR1*(see previous page for legend)* (fig001tlt).

factors, and production of reactive oxygen species. Activation of these pathways is elicited by the recruitment of various adaptor proteins to the receptor, connecting it to downstream signalling molecules (Refs 5, 6). With regard to apoptosis induction, the widely accepted view is that the adaptors TRADD ('TNFR-associated death domain protein') and FADD ('Fas-associated

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death domain protein') become recruited to the death domain, which results in the autocatalytic cleavage of pro-caspase-8. This leads to activation of the caspase cascade and in turn protein and DNA degradation, culminating in cell dismantling (Ref. 6). In parallel, recruitment of other adaptors, RIP ('receptor-interacting protein') and TRAF2 ('TNFR-associated factor 2'), mediates activation of the NF-κB transcription factor; this is viewed as an anti-apoptotic pathway (Ref. 8).

Sphingolipids: structural cell components and second messengers

Sphingolipids (so named for their apparent enigmatic, 'sphinx-like' nature) are complex lipids found in all mammalian cells and are mostly located in the plasma membrane. They all contain as a backbone a long-chain base – the sphingoid base (mostly sphingosine) – linked to a fatty acid by an amide bond, thus forming ceramide (Ref. 9) (Fig. 2). Addition of a phosphocholine substituent or sugar(s) to ceramide gives rise to the major sphingolipid sphingomyelin (SM) or to glycosphingolipids, respectively. De novo synthesis of ceramide occurs at the cytosolic face of the endoplasmic reticulum, starting by condensation of serine and palmitoyl-CoA via serine palmitoyl-transferase (Fig. 3). The resulting keto-sphinganine is reduced and *N*-acylated by ceramide synthase to form dihydroceramide, which is then desaturated to yield ceramide. Ceramide is also produced by breakdown of all sphingolipids by glycosidases (for glycolipid degradation) and sphingomyelinases (SMases; for SM degradation). These hydrolytic steps occur in the acidic organelles as well as in several other subcellular compartments. Ceramide is deacylated by ceramidase to release sphingosine, which is then phosphorylated by sphingosine kinase (Ref. 9).

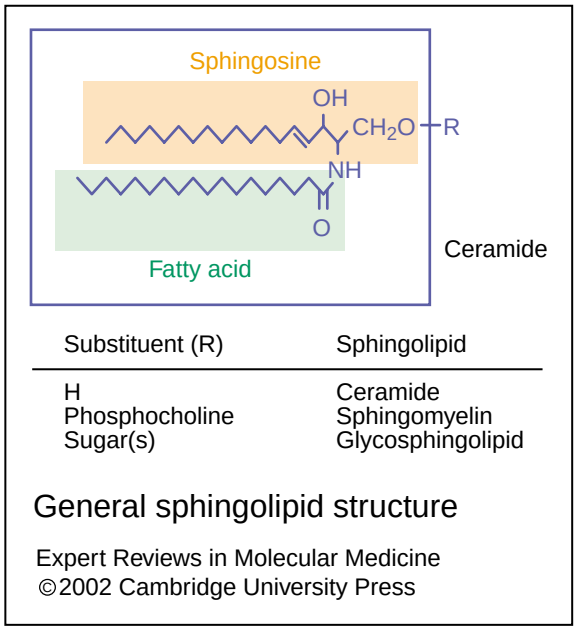

Figure 2. General sphingolipid structure. All sphingolipids contain a sphingoid long-chain base (e.g. sphingosine) that is linked to a fatty acid molecule through an amide bond, thereby forming the ceramide unit. Addition of phosphocholine or carbohydrates to ceramide leads to sphingomyelin or glycosphingolipids, respectively **(fig002tlt)**.

Several sphingolipids such as ceramide, sphingosine and sphingosine 1-phosphate are now recognised as second messengers (Refs 2, 3, 10). Ceramide, or its metabolic products, appears to mediate the effects of several extracellular stimuli including cytokines, growth factors and stress agents, leading to various effects including cell proliferation, differentiation or apoptosis (Refs 9, 11). These sphingolipids are produced upon cell treatment by these agents and, when exogenously added, can mimic their biological effects. In addition, interfering with the production of sphingolipid mediators has been shown to alter the biological response of the cell to the external stimulus (Refs 9, 11). Generation of sphingolipid mediators and the pathways they activate are conserved from yeast to humans, highlighting their importance (Ref. 9).

TNF-α-activated sphingolipid production

Early observations on agonist-stimulated generation of sphingolipids demonstrated that TNF-α was able to trigger SM degradation and ceramide generation (Ref. 12). TNF-α has now been shown to promote SM turnover and/or ceramide generation in various cell types (Table 1) as well as in vivo (Ref. 13). This production involves TNFR1 (Refs 14, 15) and occurs with differing kinetics, from minutes to hours post-treatment. These discrepancies might be related to cell-type specificity, the dose of TNF-α or, more simply, to the fact that complete time-course studies have not always been performed. Alternatively, different mechanisms might lead to increased ceramide levels.

One mechanism is SM hydrolysis. In this case, different subcellular pools of SM could serve

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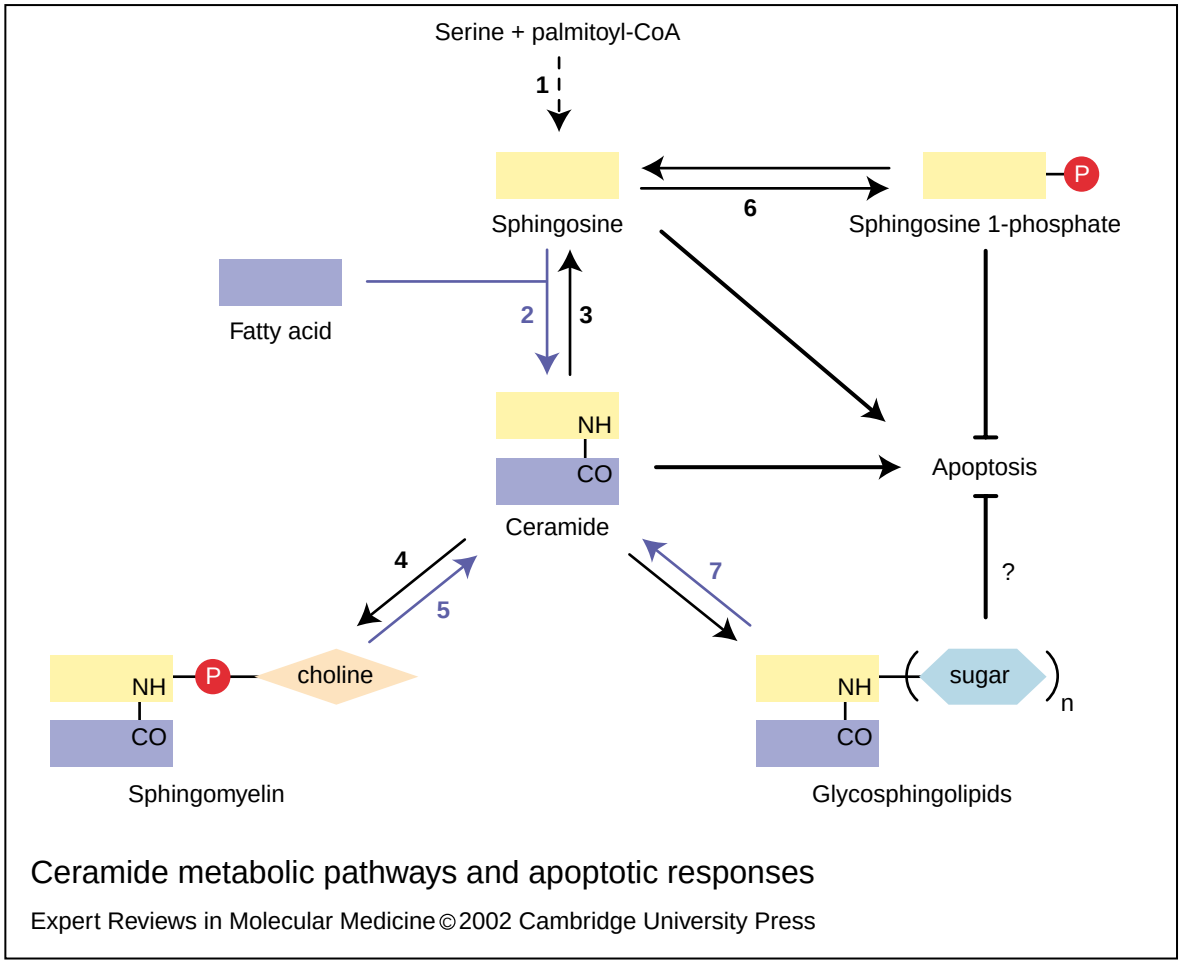

Figure 3. Ceramide metabolic pathways and apoptotic responses. The pro-apoptotic sphingolipid ceramide can be produced through three different pathways: de novo synthesis from condensation of serine with palmitoyl-CoA and acylation of the sphingoid base (enzymatic pathways 1 and 2); sphingomyelin hydrolysis (enzymatic pathway 5); or deglycosylation of glycolipids (enzymatic pathway 7). Sphingosine, which can be generated through ceramide catabolism (enzymatic pathway 3), also behaves as a pro-apoptotic lipid. By contrast, sphingosine 1-phosphate, which is derived from sphingosine (enzymatic pathway 6), antagonises apoptosis. The possible anti-apoptotic role of glycosphingolipids is not firmly established. Enzymes for numbered pathways: 1, serine palmitoyl-transferase; 2, ceramide synthase; 3, ceramidase; 4, sphingomyelin synthase; 5, sphingomyelinase; 6, sphingosine kinase; 7, glycosidase(s) **(fig003tlt)**.

as reservoirs for agonist-induced ceramide production, including acidic compartments (Ref. 16), the inner leaflet of the plasma membrane (Refs 17, 18) or caveolae (Ref. 19). SMases with an acidic pH optimum as well as SMases with a neutral pH optimum have been reported to be activated following TNF-α treatment (Ref. 10). Whereas the adaptors TRADD and FADD might mediate activation of an acid SMase, the recently described FAN ('factor associated with neutral SMase activation') protein regulates neutral SMase (see below). However, conflicting reports on the role of acid, lysosomal SMase in TNF-α-induced ceramide formation and cell death have been published following the study of responses in cells genetically deficient in lysosomal SMase (Refs 20, 21, 22).

Another mechanism that could account for ceramide production after TNF-α challenge is a stimulation of de novo synthesis, as indicated by the abrogation of ceramide accumulation in the presence of ceramide synthase inhibitors (Refs 23, 24). This de novo ceramide synthesis seems to account for the late, sustained generation of ceramide, a process that also participates in TNF-α-induced cell death.

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Table 1. Published examples of tumour necrosis factor α (TNF-α)-stimulated sphingomyelin (SM) hydrolysis, sphingomyelinase (SMase) activation and/or ceramide formation and the agents that regulate these events (tab001tlt)

Cell type	SM hydrolysis[a]	Ceramide generation[a]	SMase activation	Inhibited by	Activated by	Refs
HL-60 (human leukaemia)	30–60 min	60 min	–[b]	–	–	12
	7.5 min	7.5 min	N-SMase	–	–	59
	10 min	–	–	–	–	14
Jurkat (human leukaemia)	2 min	2 min	A-SMase	D609	–	16
U937 (human leukaemia)	5 min	2 min	A-SMase	D609	–	16
	10 min	10 min	–	–	–	31
	–	–	A-SMase, N-SMase	D609, monensin (for A-SMase)	–	60
	60 min	60 min	A-SMase, N-SMase	—	—	38
	15 min	20 min	–	–	–	61
	–	30 min	–	PMA	–	28
70Z/3 (murine pre-B)	–	2 min	A-SMase	–	–	16
Human skin fibroblast	30–60 min	30 min	N-SMase	Caveolin	FAN	15,19, 20
Rat mesangial	–	–	N-SMase	–	–	62
MCF7 (human breast cancer)	5–10 min	10–20 min	A-SMase, N-SMase	–	–	39
	–	20 h	–	CrmA, Ac-YVAD-CHO	–	37
	14 h	24 h	N-SMase	GSH, NAC, CrmA	–	47
	–	24 h	–	Bcl-x_L	–	49
	–	24 h	–	FB1, GSH	–	24
Bovine endothelial	–	3 h	–	FB1	–	23
Cos 7 (monkey kidney)	–	–	A-SMase	–	TRADD	41
HEK 293 (human embryonic kidney)	–	–	A-SMase	CrmA, Ac-YVAD-cmk	TRADD, FADD	41
Kym-1 (human rhabdo-myosarcoma)	–	2 min	N-SMase		–	63
		40 min		z-VAD-fmk		
		3 h	A-SMase			
Rat primary astrocyte	45 min	45 min	–	NAC, PDTC	–	64
Rat primary microglia	–	30 min	–	NAC	–	64

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Table 1. Published examples of tumour necrosis factor α (TNF-α)-stimulated sphingomyelin (SM) hydrolysis, sphingomyelinase (SMase) activation and/or ceramide formation and the agents that regulate these events (tab001tlt) ***(continued)***

Cell type	SM hydrolysis	Ceramide generation	SMase activation	Inhibited by	Activated by	Refs
Rat oligodendrocyte	–	45 min	–	NAC	–	64
Rat C6 glioma	–	45 min	–	NAC	–	64
Human oligodendroglioma	–	12 h	–	–	Interferon	65
Mouse embryonic fibroblast	–	–	A-SMase, N-SMase	–	FADD	42
L929 (murine fibroblast)	12 h	24 h	A-SMase, N-SMase	–	cPLA2	45
	12 h	–	–	GSH	–	46
	–	24 h	–	FB1, GSH	–	24
MC3T3E1 (murine osteoblast)	–	45 min	–	Dexamethasone	–	66
HeLa (human cervix carcinoma)	–	15 min	–	FB1	PDMP	67

[a] For each event, peak is indicated when available.
[b] Dashes indicate information is not available.

Abbreviations: A-SMase, acid sphingomyelinase; cPLA2, cytosolic phospholipase A2; CrmA, cytokine response modifier A; FADD, Fas-associated death domain protein; FAN, factor associated with neutral sphingomyelinase activation; FB1, fumonisin B1; GSH, glutathione; NAC, *N*-acetylcysteine; N-SMase, neutral sphingomyelinase; PDMP, 1-phenyl-2-decanoylamino-3-morpholino-1-propanol; PDTC, pyrrolidinedithiocarbamate; TRADD, TNF receptor (TNFR)-associated death domain protein; PMA, phorbol 12-myristate 13-acetate; Ac-YVAD-CHO, Ac-Tyr-Val-Ala-Asp-aldehyde; Ac-YVAD-cmk, Ac-Tyr-Val-Ala-Asp-chloromethylketone; z-VAD-fmk, benzyloxycarbonyl-Val-Ala-Asp-fluoromethylketone.

Sphingosine generation was shown to accompany and mediate the cytotoxic effect of TNF-α in various cell lines (Refs 25, 26, 27). In U937 leukaemic cells, TNF-α might reduce survival by decreasing sphingosine kinase activity and, consequently, intracellular levels of the anti-apoptotic sphingolipid sphingosine 1-phosphate (Ref. 28). In other cell types where TNF-α does not induce apoptosis but other endpoints, the cytokine instead stimulates sphingosine kinase, thereby protecting cells against death (Ref. 29). The anti-apoptotic function of sphingosine 1-phosphate is believed to be exerted by both intracellular and extracellular [endothelial differentiation gene (EDG) receptor-mediated] actions (Refs 9, 11, 28).

Thus, experimental evidence has accumulated to show that treatment of mammalian cells with TNF-α transiently affects sphingolipid metabolism by stimulating SM hydrolysis and ceramide formation. Furthermore, ligation of other members of the TNFR family, including CD40, CD95, the p75NGF ('nerve growth factor') and TRAIL ('TNF-related apoptosis-inducing ligand') receptors, induces apoptosis and engages sphingolipid second messengers (Refs 9, 10, 30). Finally, although not reviewed here, sphingolipids appear to mediate other biological effects of TNF-α (e.g. monocytic differentiation, fibroblast proliferation, expression of adhesion molecules, and insulin resistance) in addition to cell death induction (Refs 11, 30).

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Role of sphingolipids in TNF-α-induced cell death

Since the seminal study of Obeid et al. on TNF-α-induced death of leukaemic cells (Ref. 31), a wealth of reports on the role of ceramide and other sphingolipids in the regulation of apoptosis have been published. These have attempted to elucidate both the mechanism(s) of action of sphingolipids and their place in apoptosis signalling. For detailed information on the cellular targets that are regulated by sphingolipids during apoptosis in general, the reader is referred to recent reviews (Refs 3, 30).

To summarise, both ceramide and sphingosine (Ref. 32) activate effector ('executioner') caspases through disruption of mitochondrial functions and release of apoptogenic mitochondrial factors such as cytochrome *c* and Smac/DIABLO ('second mitochondria-derived activator of caspase'/'direct IAP binding protein with low pI') (Ref. 33). Among the primary targets believed to couple ceramide and downstream effectors or modulators of apoptosis are serine/threonine protein phosphatases such as ceramide-activated protein phosphatase 2A (CAPP), proline-directed kinases such as KSR ('kinase suppressor of Ras'), c-Jun N-terminal kinases (JNKs), the protein kinase Cζ isoform and the aspartyl protease cathepsin D (Refs 3, 9, 30). With regard to the interaction of sphingolipids with members of the MAPK ('mitogen-activated protein kinase') family, ceramide has been reported to mediate the activation of both JNK and ERK ('extracellular regulated kinase'), which are usually associated with apoptosis and growth (or inflammatory) signals, respectively (Refs 2, 30). A closer examination of published data indicates that neutral SMase-derived ceramide does not mediate TNF-α-induced ERK activation, whereas sphingosine 1-phosphate stimulates ERK and inhibits JNK (Refs 15, 28). Ceramide also activates the nuclear translocation of NF-κB (Ref. 16); this effect, however, remains controversial (Ref. 11). Moreover, TNF-α-induced activation of NF-κB appears to proceed independently of acid SMase (Refs 34, 35, 36).

Are sphingolipid mediators key players in TNF-α-induced apoptosis? The evidence in favour of this is as follows. First, an increase in ceramide content is not the trivial consequence of cell collapse, as indicated by the finding that overexpression of anti-apoptotic proteins (e.g. Bcl-2 or Bcl-x_L) prevents cell death without affecting ceramide generation (Ref. 37). This suggests that ceramide production indeed precedes late apoptotic events. Second, ceramide production is blocked in cells resistant to the cytotoxic effect of TNF-α (Refs 27, 38, 39), but addition of exogenous ceramide to, or restoration of ceramide formation in, resistant cells leads to apoptosis induction (Ref. 40). Third, an inherited deficiency of acid SMase activity has been reported by some authors (Ref. 22), but not others (Ref. 21), to partially impair apoptosis induction. Finally, blocking neutral SMase activation by obliteration of the functions of the adaptor protein FAN (see below) reduces TNF-α-induced cell death (Ref. 15). Thus, sphingolipid metabolites appear to contribute (at least in part) to the cellular mechanisms involved in TNF-α-induced cell killing.

Regulation of sphingolipid production by TNF-α

Several pathways that regulate TNF-α-stimulated sphingolipid generation and onset of apoptosis have been identified. Regarding ceramide production by SM hydrolysis, various proteins belonging to the TNF-α-initiated signalling cascades can modulate SMase activation. The following working model can be proposed (Fig. 4). After activation of TNFR1 by binding of TNF-α, the adaptor proteins TRADD and FADD stimulate acid SMase (Refs 41, 42), whereas FAN is required for neutral SMase activation (Ref. 43); by contrast, the adaptors TRAF2 and RIP do not affect acid SMase (Ref. 41). FAN is a 917 amino acid protein that binds to a short domain of TNFR1 (Ref. 44). Abrogation of FAN function by overexpression of an N-terminally deleted FAN mutant (which exhibits a dominant-negative effect) or by gene knockout results in abrogation of TNF-α-induced SM hydrolysis, suppression of the subsequent ceramide formation, and inhibition of cell death (Ref. 15).

Downstream of the TNFR and its adaptors, other players regulate SM breakdown. These include some proteases [inhibitable by 'cytokine response modifier A' (CrmA)] (Ref. 37), phospholipase A2 and its product arachidonic acid (Refs 45, 46), phospholipase C and diacylglycerol (Ref. 16), glutathione and reactive oxygen species (Ref. 47), phosphoinositide 3-kinase (Ref. 48), and protein kinase C (Ref. 10). Manipulation of these pathways impairs ceramide generation and induction of cell death.

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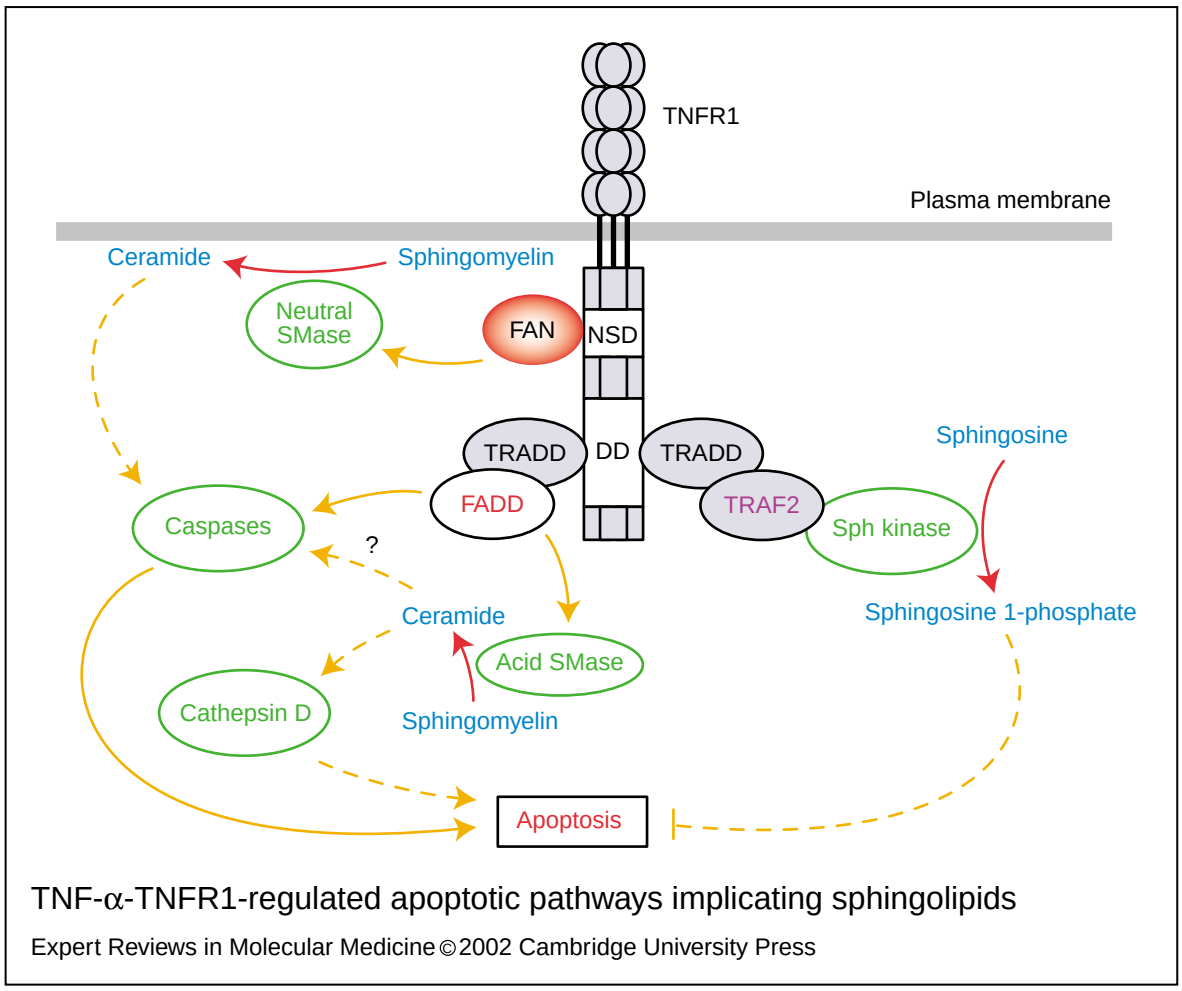

Figure 4. TNF-α–TNFR1-regulated apoptotic pathways implicating sphingolipids. Activation of a neutral sphingomyelinase (SMase) is mediated by the adaptor FAN, which binds to a membrane-proximal domain of the cytoplasmic region of TNFR1 (tumour necrosis factor receptor 1), called NSD ('neutral sphingomyelinase domain'). The ceramide generated by this FAN-regulated SMase contributes to caspase activation. Ceramide can also be produced via the stimulation of an acid SMase through the adaptors TRADD, which binds to the death domain (DD) of TNFR1, and FADD; this ceramide might activate the aspartyl protease cathepsin D (activation of caspases by acid-SMase-derived ceramide is less well established). Interaction of sphingosine kinase (Sph kinase) with TRAF2 might promote the formation of the anti-apoptotic sphingolipid mediator sphingosine 1-phosphate. Dashed lines depict indirect pathways. FADD, Fas-associated death domain protein; FAN, factor associated with neutral sphingomyelinase activation; TRADD, TNFR-associated death domain protein; TRAF2, TNFR-associated factor 2 **(fig004tlt)**.

Overexpression of the anti-apoptotic Bcl-2 member Bcl-x_L has also been reported to abrogate the late TNF-α-induced ceramide accumulation in breast cancer cells (Ref. 49), possibly suggesting that the post-mitochondrial increase in ceramide further amplifies the apoptotic response.

With regard to sphingosine kinase activation by TNF-α, very recent data indicate that the adaptor TRAF2 interacts with and stimulates this enzyme (Ref. 50). This links the essential role of TRAF2 in anti-apoptosis and the generation of the anti-apoptotic and mitogenic lipid sphingosine 1-phosphate.

Clinical implications

Several pathological conditions in humans are linked to, or associated with, alterations in the TNF-α–TNFR interaction, and are often a consequence of abnormal production or secretion of TNF-α (Refs 51, 52). The genetic condition

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TRAPS ('TNFR-associated periodic syndrome'), characterised by a periodic fever, involves dominant mutations in the gene encoding TNFR1 that result in reduced serum levels of soluble TNFR, leading to inflammation due to unopposed TNF-α action (Ref. 52). TNF-α-induced cytotoxicity might underlie (at least in part) the pathophysiology of other disease states such as septic shock and various autoimmune disorders including rheumatoid arthritis, some forms of insulin-dependent diabetes mellitus, multiple sclerosis and Crohn's disease (Refs 51, 52). Accordingly, strategies aimed at blocking TNFR activation have offered substantial therapeutic effects (Refs 53, 54). These approaches include antibodies against TNF-α (infliximab) or soluble TNFR fusion proteins (etanercept), which either block the activity of or 'mop-up' TNF-α, respectively. For rheumatoid arthritis and inflammatory bowel disease, these TNF-α-targeting agents have proven to be effective in correcting disease parameters and ameliorating symptoms. However, because anti-TNF-α or soluble TNFR molecules are delivered systemically, they also act on cells and tissues that are not involved in the disease, which might have some adverse effects. In addition, the converse strategy, that of administering TNF-α to eradicate malignant cells, is also not employed because of deleterious effects related to the cytokine action on non-cancer cells. It is thus desirable to develop approaches that are more tissue-specific.

One possibility for the basis of a tissue-specific approach is to target intracellular sphingolipid metabolism in a particular organ or cell type. Thus, whereas intracellular accumulation of a pro-apoptotic lipid messenger (e.g. ceramide) might eliminate an unwanted cell, decreasing the level of this toxic mediator or increasing that of a sphingolipid acting as a survival/resistance factor (e.g. sphingosine 1-phosphate or glucosylceramide) might protect cells from the cytotoxic action of TNF-α. Such strategies have already been successfully tested in the killing of tumour cells (Refs 55, 56) as well as in preventing radiation-induced cell death of oocytes (Ref. 57). In the former situation, inhibitors of ceramidase, sphingosine kinase or glucosylceramide synthase were employed; by contrast, the latter strategy used in vivo therapy with sphingosine 1-phosphate. Therefore, it is conceivable that similar modalities could be applied to cancer and inflammatory or autoimmune diseases involving TNF-α. Although sphingolipid-based clinical trials in these particular disorders remain distant, sphingolipid analogues and enzymes of sphingolipid metabolism represent attractive tools and targets for alternative modulation of TNF-α effects and combination therapies.

Concluding remarks

Evidence has accumulated that in human (and murine) cells TNF-α elicits profound changes in sphingolipid metabolism, and that generation of these potential lipid second messengers can mediate some of the biological responses to TNF-α. Indeed, various actions of TNF-α can be mimicked and mediated by ceramide, sphingosine or sphingosine 1-phosphate. These responses include cell differentiation, interleukin secretion, expression of adhesion molecules and induction of apoptosis (Ref. 11). Although the contribution of ceramide to apoptosis has been debated (Ref. 58), this sphingolipid can be viewed as one of the players in TNF-α-induced cell death signalling. Whether these lipids, the production of which appears tightly regulated, act as authentic second messengers in TNF-α signalling of apoptosis, either as 'helpers' (through local changes in membrane structure and properties) or as 'amplifiers' (cooperating with other mediators to ultimately result in a full apoptotic response), still remains to be resolved. It is expected that in the near future some important tools, such as specific inhibitors of enzymes of sphingolipid metabolism and animals harbouring a genetic defect in these enzymes, will become available, providing new insights into the precise function of sphingolipids in (TNF-α-mediated) apoptosis induction.

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Further resources, reading and contacts

A complete review on sphingolipid biochemistry and biology:

Huwiler, A. et al. (2000) Physiology and pathophysiology of sphingolipid metabolism and signaling. Biochim Biophys Acta 1485, 63-99, PubMed: 20292871

An updated view of enzymes of ceramide metabolism, ceramide targets, ceramide signalling and cross-talk with other signalling pathways, including therapeutic implications and techniques for ceramide analysis:

Futerman, T. (2002) Ceramide Signaling, Landes Bioscience

A review on the structure and roles of proteins of the TNF–TNFR superfamily:

Locksley, R.M., Killeen, N. and Lenardo, M.J. (2001) The TNF and TNF receptor superfamilies: integrating mammalian biology. Cell 104, 487-501, PubMed: 21135272

Two recent reviews on the signalling pathways triggered by TNF-α:

Baud, V. and Karin, M. (2001) Signal transduction by tumor necrosis factor and its relatives. Trends Cell Biol 11, 372-377, PubMed: 21406283

MacEwan, D.J. (2002) TNF receptor subtype signalling: differences and cellular consequences. Cell Signal 14, 477-492, PubMed: 21895300

Related articles in *Expert Reviews in Molecular Medicine*:

O'Connell, J. (2001) Role of Fas–FasL in inflammatory diseases. Exp. Rev. Mol. Med. 10 December, http://www-ermm.cbcu.cam.ac.uk/01003969h.htm

Fantini, J. et al. (2002) Lipid rafts: structure, function and role in HIV, Alzheimer's and prion diseases. Exp. Rev. Mol. Med. 20 December, http://www.expertreviews.org/02005392h.htm

Further information on apoptosis is provided by the following websites:

http://www.apoptosis-db.org
http://vlib.org/Science/Cell_Biology/apoptosis.shtml
http://www.celldeath.de/mainfram.htm

Images of cells undergoing apoptosis can be found at the Cells alive! website:

http://www.cellsalive.com

Features associated with this article

Figures

Figure 1. Signalling pathways regulated by TNF-α–TNFR1 (fig001tlt).
Figure 2. General sphingolipid structure (fig002tlt).
Figure 3. Ceramide metabolic pathways and apoptotic responses (fig003tlt).
Figure 4. TNF-α–TNFR1-regulated apoptotic pathways implicating sphingolipids (fig004tlt).

Table

Table 1. Published examples of tumour necrosis factor α (TNF-α)-stimulated sphingomyelin (SM) hydrolysis, sphingomyelinase (SMase) activation and/or ceramide formation and the agents that regulate these events (tab001tlt).

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