Human umbilical vein endothelial cells (HUVEC), like most normal cells, are resistant to tumor necrosis factor-α (TNF)-induced apoptosis in spite of TNF activating sphingomyelinase and generating ceramide, a known inducer of apoptosis. Here we report that TNF activates another key enzyme, sphingosine kinase (SphK), in the sphingomyelin metabolic pathway resulting in production of sphingosine-1-phosphate (SIP) and that SIP is a potent antagonist of TNF-mediated apoptosis. The TNF-induced SphK activation is independent of sphingomyelinase and ceramidase activities, suggesting that TNF affects this enzyme directly rather than through a mass effect on sphingomyelin degradation. In contrast to normal HUVEC, in a spontaneously transformed endothelial cell line (C11) TNF stimulation failed to activate SphK and induced apoptosis as characterized by morphological and biochemical criteria. Addition of exogenous SIP or increasing endogenous SIP by phorbol ester markedly protected C11 cell line from TNF-induced apoptosis. Conversely, N,N-dimethylsphingosine, an inhibitor of SphK, profoundly sensitized normal HUVEC to killing by TNF. Thus, we demonstrate that the activation of SphK by TNF is an important signaling for protection from the apoptotic effect of TNF in endothelial cells.

Tumor necrosis factor-α (TNF),¹ originally defined by its tumoricidal activity, is a pleiotropic cytokine that has strikingly different biologic effects in different cell types (1, 2). Although TNF elicits a cytotoxic effect on numerous tumor cells or virally infected cells, most normal cells are resistant (3, 4). For example, human endothelial cells that play a crucial role in maintaining normal vasculature and modulating the inflammatory response are not directly killed by TNF (4, 5). However, endothelial cells as well as other cells can be rendered sensitive to TNF in the presence of protein or RNA synthesis inhibitors such as cycloheximide or actinomycin D (3, 4). Conversely, it has been demonstrated that sensitive cells can be made resistant to TNF challenge by prior sublethal exposure to TNF (6). These findings have led to the hypothesis that TNF-inducible signals confer a protective effect from the cytotoxic activity of TNF in normal cells.

A major advance in understanding TNF signaling was the identification of protein molecules that are recruited to TNF receptor-1 (p55) and receptor-2 (p75) following ligand-induced trimerization (7–13). Engagement of the TNF receptor results in recruitment of a complex of proteins to the cell membrane including TRADD, FADD/MORT1, and RIP, which may lead to the further recruitment and activation of various caspases and, subsequently, to cell death (9, 10). On the other hand, TNF induces the interaction of its receptor with a second class of adaptor protein TRAFs and recruits downstream signals such as NF-κB-inducing kinase to activate NF-κB, which protects many different cell types from death (10–13). Additional signaling molecules, sphingolipids, have recently emerged as regulators of cell growth, differentiation, diverse cell phenotypes, and cell death (14–20). Signaling via sphingolipid turnover is exemplified by two distinct pathways: the formation of ceramide resulting from the activation of sphingomyelinase by TNF and a variety of other stimuli (14–16) and the formation of sphingosine-1-phosphate (SIP) upon sphingosine kinase (SphK) activation by several growth factors such as platelet-derived growth factor and phorbol ester (17–19). Thus, it has been proposed that cells activate sphingomyelinase in response to cytokines, whereas growth factors activate SphK and thereby choose between the formation of ceramide that favors cell death versus SIP that inhibits death (19). However, how the activities of these key enzymes are controlled in response to various stimuli is still incompletely understood.

In this study we report that in human endothelial cells TNF itself can activate SphK independent of sphingomyelinase activation to generate SIP that acts as a protective factor against the cytotoxic effect of TNF. In addition, we identify a transformed endothelial cell line (C11) that undergoes apoptosis in response to TNF stimulation and that exhibits a specific defect in SphK activation. Thus, our data demonstrate that in human endothelial cells TNF simultaneously and independently activates two antagonistic biochemical signaling pathways, sphingomyelinase and SphK pathways, the balance of which could regulate the fate of cell in response to TNF stimulation.

EXPERIMENTAL PROCEDURES

Materials—TNF was purchased from R & D Systems Inc. (Minneapolis, MN). C2-ceramide, SIP, sphingosine, N,N-dimethylsphingosine (DMS), and dihydro sphingosine were from Biomol (Plymouth Meeting, PA). [3H]Serine and [choline-methyl-14C]phosphatidylcholine were from NEN Life Science Products. γ-[32P]ATP was purchased from Bresatec (Adelaide, Australia). Escherichia coli diacylglycerol kinase was from Calbiochem (La Jolla, CA). Anti-CPP32 antibody was purchased from Transduction Laboratories. Other chemicals were from Sigma.

Cell Culture—HUVEC were isolated as described previously (21).
The C11 cell line was generated in our laboratory and characterized as previously reported (22). All cells were cultured on gelatin-coated culture flasks in medium M199 with Earle’s salts supplemented with 20% fetal calf serum, endothelial growth supplement (Collaborative Research), and heparin. Niemann-Pick type A skin fibroblasts and normal skin fibroblasts were kindly provided by Dr. E. Reith (Children’s Hospital, Adelaide, Australia) and cultured in medium M199 with Earle’s salts supplemented with 10% fetal calf serum.

**Apoptosis Assays—**Oligonucleosomal banding was demonstrated by harvesting total cellular DNA. After the indicated treatment both adherent and detached cells were harvested, washed with phosphate-buffered saline (PBS), and lysed in 50 mM Tris-HCl, pH 7.5, for 10 min at 0.5% Triton X-100, and 0.5 mM/ml proteinase K for 16 h at 50°C. Samples were then extracted with phenol/chloroform/isoamyl alcohol and precipitated with ethanol. The pellet was resuspended in Tris/EDTA and 10 μg/ml RNase A, and the DNA was separated by electrophoresis on a 1.8% agarose gel stained with ethidium bromide.

**Apoptosis was also assessed by in situ staining cells based on terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) using a cell death detection kit (Roche Molecular Biochemicals). The labeling procedure was performed in Lab-Tek 8-well chamber slides following the supplier’s instructions. After staining, cells were analyzed under fluorescence microscopy (Olympus BH2).**

**Cell Viability Assay—**Cell viability was measured using MTT dye reduction assay. Cells were seeded on 48-well plates at a density of 30,000 cell/well for 24 h, and the cells reached confluence. After various treatments, cells were incubated with 1 mg/ml MTT for 4 h. The medium was then aspirated, and the formazan product was solubilized with 10% SDS in 10 mM HCl. Cell viability was assessed by spectrophotometry at 570 and 650 nm absorbance in a 96-well enzyme-linked immunosorbent assay reader plate.

**Ceramide Measurement—**For ceramide-labeled cells, the cells were incubated with regular growth medium containing [3H]serine (10 μCi/ml) in the presence of 0.5 mM 4-deoxypyridoxine and 0.1 mM L-canaline. After 48 h of incubation, the radioactive medium was removed, and cells were incubated for another 4 h in culture medium. Cellular lipids were extracted and resolved by TLC with two different solvent systems as described previously (20). Radiolabeled sphingolipid spots, identified by comparison with concomitantly run standards, were scraped and quantitated by scintillation spectrometry. The levels of sphingolipids were normalized by radioactivity recovered in total cellular lipids.

**Ceramide Measurement—**After treatment for the indicated times, cells were harvested, and lipids were extracted with chloroform/methanol (2:1) and resolved by TLC with an organic phase extract containing 15 mM EDTA. Cellular ceramide was quantified with the diacylglycerol kinase reaction as described previously (14). Briefly, the lipids in the organic phase extract were dried and resuspended into sample buffer containing 7.5% n-octyl-β-D-glucopyranoside, 5 mM CaCl2, and 1 mM dTTP. The samples were reacted with diacylglycerol kinase and [γ-32P]ATP in enzyme buffer containing 20 mM Tris/HCl, pH 7.4, 1 mM MgCl2, and 15% glycerol. After 30 min at 22°C, the reaction was stopped by extraction of lipids. The product of the phosphorylation reaction, ceramide-1-phosphate was resolved by TLC using CHCl3/CH3OH/H2O (65:15:5) as solvent, detected, and quantified by the Phosphoimager system (Molecular Dynamics). To exclude a possible error caused by some factors in the extracts affecting diacylglycerol kinase (23), synthetic C2-ceramide was added in assays as an internal control. There were no changes in the phosphorylated C2-ceramide in this assay system.

**Measurement of SphK Activity—**As described previously (18, 20), cells were washed with ice-cold PBS and homogenized in lysis buffer (100 mM phosphate buffer, pH 7.2, 10 mM MgCl2, 20% glycerol, 1 mM diithiothreitol, 1 mM EDTA, 20 μM ZnCl2, 1 mM Na3VO4, 15 mM NaF, 10 μg/ml leupeptin and aprotinin, 1 mM phenylmethylsulfonyl fluoride, 10 μg/ml leupeptin and aprotinin. The lysates were then centrifuged at 500 × g for 5 min to remove the debris and then ultracentrifuged at 100,000 × g for 1 h at 4°C to pellet the membrane fraction. The membrane pellets were resuspended in a reaction buffer supplemented with 1% Triton X-100 containing either 0.1 μM CAPSO (3-cyclohexylamino-2-hydroxyl-1-propanesulfonic acid), pH 9, or 0.1 M acetate buffer, pH 4.5. After incubation with 0.2 μCi of [3H]-oleoyl-sphingosine (ceramide) for 1 h at 37°C, the reaction was terminated by extraction of oleic acid. The labeled oleic acid was resolved by TLC and quantitated by liquid scintillation counting.

**Immunoblot Analysis—**Cytosolic proteins (30 μg) were resolved by 15% SDS-polyacrylamide gel electrophoresis and transferred to nitrocellulose filters (Schleicher & Schuell). Membranes were incubated with 0.2% (w/v) Tween 20 and 5% (w/v) nonfat dry milk in Tris-buffered saline solution (TPBS) (0.02 M Tris-HCl, pH 7.4, 140 mM NaCl, and 0.05% Tween 20). Radiolabeled IgGs were detected by incubation with secondary antibodies conjugated to horseradish peroxidase and developed using ECL system (Amersham Pharmacia Biotech).

**RESULTS**

**TNF Does Not Induce Apoptosis in Normal HUVEC but It Does in C11 Cell Line**—In agreement with previous reports that HUVEC are resistant to the cytotoxic effect of TNF (4, 5), treatment of HUVEC with up to a saturating dose (10 ng/ml) of TNF resulted in no morphological changes of death (Fig. 1A, a and b). However, TNF-induced cell death was observed morphologically as early as 6 h poststimulation (Fig. 1A, c and d) in a spontaneously transformed cell line generated from HUVEC, named C11, which has maintained many other functional endothelial characteristics (22). The electrophoretic pattern of the DNA extracted from the treated C11 cell line demonstrated the typical DNA internucleosomal fragmentation of apoptotic cells (Fig. 1B). To quantify the number of cells undergoing apoptosis, the MTT dye reduction assay was performed. Again, HUVEC displayed resistance to TNF cytotoxic effects, although these cells undergo apoptosis with serum deprivation (Fig. 1C). By contrast, in the C11 cell line TNF-induced cell death in a dose-dependent manner in the cultures containing various concentration of serum (Fig. 1C). Thus, the difference in phenotype between HUVEC and C11 cell line provided a useful model to investigate the mechanism for endothelial cell death in response to TNF.

**TNF-induced Ceramide Generation Is Not Sufficient to Trigger Apoptosis in Endothelial Cells**—Ceramide, generated from sphingomyelin hydrolysis, has emerged as a second messenger in mediating apoptosis induced by various stimuli including TNF (14-16), although the role of ceramide is still controversial. As shown in Fig. 2A, the addition of exogenous ceramide, cell-permeable C2-ceramide, was able to induce apoptosis in both HUVEC and C11 cell line in a dose-dependent manner. In addition, treatment with sphingomyelinase (1 unit/ml) to gen-
erate endogenous ceramide by hydrolysis of sphingomyelin also induced apoptosis in both endothelial cell types (Fig. 2A, inset). These data raise the hypothesis that the resistance to TNF cytotoxic effect in HUVEC may be due to a lack of ceramide generation in response to TNF. However, our previous report showed that TNF rapidly induced an increase in intracellular ceramide levels (2-fold increase) peaking at 30 min after stimulation and a concomitant decrease in sphingomyelin content (20). To confirm this finding, Fig. 2B shows that TNF induced an identical sphingomyelin-ceramide turnover in both HUVEC and C11 cell lines. There was also no difference in the basal levels of sphingomyelin and ceramide in these two cell lines. Thus, ceramide itself could not explain the differential responses to TNF between HUVEC and C11 cell line.

Resistance to TNF Cytotoxic Effect Is Associated with SphK Activation—S1P, another sphingomyelin metabolite generated from sphingosine through SphK activation, has been implicated as a signal molecule in mediating cell growth, proliferation, and protecting cell death (17–19). To explore the role of SphK in regulating TNF cytotoxic effect, the activity of SphK was measured. Treatment of HUVEC with TNF caused a rapid and transient increase in SphK activity reaching a maximum of 174 ± 616% (p = 0.01) of basal within 10 min (Fig. 3), which was consistent with our previous report (20). In contrast, TNF failed to induce any increases in SphK activity in C11 cell line. There were no significant differences in the basal levels of SphK activity between HUVEC and C11 cell line (21.4 ± 1.8 and 19.7 ± 1.9 pmol/min/mg protein, respectively). As a control, PMA (phorbol 12-myristate 13-acetate), an activator of SphK through protein kinase C activation (19), induced a similar increase in SphK activity in C11 cell line and HUVEC (Fig. 3), suggesting a specific defect in activation of SphK by TNF in C11 cell line. In parallel with the SphK activity, the production of S1P in vivo and its levels in intact cells were increased by
TNF stimulation in HUVEC (20), but not in C11 cells (data not shown), confirming the differential responses in SphK activation by TNF between these two types of endothelial cells. These data suggested that the responses of endothelial cells to the TNF cytotoxic effect might be associated with the SphK activation.

**TNF-induced SphK Activation Is Independent of Sphingomyelinase and Ceramide Activities—**Because S1P is a downstream metabolite in ceramide metabolic pathway, the TNF-induced increases in S1P generation could be related to the activation of sphingomyelinase by TNF stimulation. To test whether the TNF-induced S1P generation is dependent or independent of sphingomyelinase activity, we studied the skin fibroblasts from a patient with Niemann-Pick disease type A, a lysosomal storage disease characterized by a complete lack of acid sphingomyelinase activity and sphingomyelin accumulation (27). The phenotype of this cell line was identified by measuring sphingomyelinase activity in neutral and acidic pH ranges in post-nuclear extracts from the Niemann-Pick fibroblasts and from age-matched normal skin fibroblasts. In comparison with normal skin fibroblasts, the Niemann-Pick fibroblast line completely lacked acidic sphingomyelinase activity and had only a small amount of neutral sphingomyelinase activity (Fig. 4A). Despite a complete lack of ceramide generation in response to TNF in the Niemann-Pick fibroblasts, the TNF-induced SphK activation and S1P generation remained unimpaired as compared with controls (Fig. 4B). Thus, it is unlikely that TNF-induced S1P generation is a downstream metabolic event of ceramide.

To further define the TNF-induced generation of S1P, we investigated ceramidase, another key metabolic enzyme upstream of S1P, which catalyzes the metabolism of ceramide to sphingosine, the precursor of S1P production. Because two main isoforms of ceramidase, acid and alkaline forms, exist in most tissues and cells (25), we used the two isoform inhibitors, N-oleylethanolamine (NOE) and (1S,2R)-O-erythro-2-(N-myristoylamino)-1-phenyl-1-propanol (MAPP). NOE is a more potent inhibitor of acid ceramidase (28), whereas MAPP predominantly inhibits the alkaline form (29). Neither NOE nor MAPP has a detectable effect in interfering with the TNF-induced increase in S1P formation in HUVEC (Fig. 5A), whereas the acid or alkaline ceramidase activity was inhibited by −60 or −70%, respectively (data not shown). As a control, TNF-induced S1P generation was completely inhibited by the addition of the competitive inhibitors of SphK, DMS or dihydro sphingosine (19, 20), suggesting a direct effect of TNF on SphK activation. In addition, Fig. 5B shows that TNF treatment did not stimulate ceramidase activity at either acidic or alkaline pH values, which are consistent with a previous report on mesangial cells (26). These data further indicate that the activation of SphK by TNF is independent of ceramidase activity.

An Inhibitor of SphK Sensitizes HUVEC to Apoptosis while S1P Protects C11 Cells from Death—**Given the potential relationship between SphK activation and cellular responses to TNF killing effect, we determined the role of SphK in resistance to TNF by using a competitive inhibitor of SphK, DMS. As shown in previous observation in Jurkat T cell line (33). In contrast, TNF failed to activate CPP32 caspase in normal HUVEC, whereas inhibition of SphK activity by DMS sensitized TNF-induced activation of CPP32 (Fig. 7C). These findings further ascertained the role of TNF-induced SphK activation in regulating cell death in human endothelial cells.

**DISCUSSION**

The endothelial cell is the interface between blood and tissue and plays an important role in organ physiology and pathology of a variety of disease states. Endothelium in normal tissues, although metabolically active, is generally considered to be quiescent because the turnover is very low (34). This would suggest that normal endothelium in the steady state has mechanisms of maintaining cell numbers by promoting viability independent of proliferation. In uninjured tissue, the endothelium also acts as a barrier to conceal the thrombogenic substratum from circulating platelets and clotting factors (35, 36). Therefore, intact endothelium plays a crucial role as a sensor and effector of signals as well as in maintaining a nonthrombogenic surface. Given the importance of an intact, viable endothelium, we attempted to study the mechanism of resistance to TNF cytotoxicity in normal endothelial cells.

The central finding of this report is that TNF not only induces sphingomyelin hydrolysis resulting in ceramide accumulation but also activates SphK to generate S1P that serves as an anti-apoptotic molecule for cell survival in normal HUVEC. We found that: (i) TNF stimulation caused sphingomyelin...
activated by PMA, an established agonist of SphK, through the activation of protein kinase C (19). Addition of exogenous S1P or treatment with PMA to generate endogenous S1P profoundly protected C11 cell line from apoptosis induced by TNF. In agreement with previous report (19), the effect of PMA on antagonizing the TFN-induced apoptosis was marked by the addition of DMS (data not shown), indicating a role of SphK activation in protecting cell death. Taken together, these data suggested that the activation of SphK after TNF-induced sphingomyelin-ceramide turnover “switches on” the signaling that could inhibit cell death.

Ceramide has been described as a mediator of apoptotic cell death in response to a variety of stimuli including TNF (14–16), and this notion has recently been challenged by other observations (see review in Ref. 37). Ceramide is also a substrate for ceramidase to form sphingosine, which can further convert to S1P via the activation of SphK. It has been reported that various mitogenic and growth factors such as PMA and platelet-derived growth factor stimulated proliferation is mediated, at least in part, by activating ceramidase (26) or SphK (17–19). Here we show for the first time that TNF itself is able to activate SphK independently of sphingomyelinase or ceramidase activation as judged by four criteria. First, TNF induced SphK activation rather than a downstream event in the sphingomyelin metabolic pathways. 

The phenomenon that the individual enzymes in sphingomyelin metabolic pathways can be regulated by a given agonist (TNF) concurs with the previous observation that interleukin-1

**Fig. 4.** TNF activated SphK independently of sphingomyelinase. A, sphingomyelinase activities were measured in acidic (ASM) and neutral (NSM) pH ranges in post-nuclear extracts from the Niemann-Pick fibroblasts (NP-SF) and from age-matched normal skin fibroblasts. B, ceramide (C) and S1P (D) levels were measured in the [3H]serine-labeled Niemann-Pick fibroblasts treated with TNF (1 ng/ml) for various times as described under “Experimental Procedures.” The results represent mean values ± S.D. from three independent experiments.

**Fig. 5.** TNF activated SphK independently of ceramidase. A, HUVEC were pretreated with a vehicle (Nil), NOE (0.5 mM), MAPP (5 μM), DMS (5 μM), and DMS (5 μM) for 30 min, respectively, followed by stimulation with (black bars) or without (gray bars) TNF (1 ng/ml) for 10 min. S1P formation in vivo was measured in the permeabilized cells. B, after cells were stimulated with TNF (1 ng/ml) or PMA (100 ng/ml) for 10 min, cellular ceramidase activity was measured at acidic or alkaline pH value. The results represent mean values ± S.D. from three independent experiments. *p < 0.001 compared with TNF stimulation.

**Fig. 6.** Effect of SphK on TNF-induced apoptosis. HUVEC (a–f) or C11 cells (g–l) were grown in 8-well chamber slides. After 24-h cultures, cells were treated with or without TNF (1 ng/ml) for 16 h in the presence of a vehicle (Nil), DMS (5 μM), S1P (5 μM, c, i, and l), or DMS + S1P (g), respectively. Apoptotic cells were assessed by in situ staining cells using TUNEL (magnification, ×400).

breakdown with a concomitant increase in ceramide; (ii) TNF induced a rapid and transient activation of SphK, with corresponding increases in S1P levels; (iii) the inhibition of S1P production by DMS, a competitive inhibitor of SphK, significantly sensitized HUVEC to TNF-induced apoptosis; and (iv) the effect of DMS was reversed by addition of S1P. These findings provided new evidence to support the hypothesis of “self-control”: that TNF itself can induce the generation of molecules that protect cells from the cytotoxicity of TNF (3).

In contrast to normal endothelial cells, the transformed C11 cell line failed to activate SphK generating S1P and underwent apoptosis in response to TNF stimulation. The defect in SphK activation is not due to the lack of this enzyme because it can be
FIG. 7. DMS sensitizes HUVEC to apoptosis, whereas S1P protects C11 cells from death. A, confluent cells were treated with or without TNF (1 ng/ml) for 16 h in the presence of vehicle or DMS (2.5, 5, and 10 μM; for HUVEC) or S1P (2.5, 5, and 10 μM; for C11). Total cellular DNA was then isolated and analyzed by electrophoresis on a 1.8% agarose gel stained with ethidium bromide. Results are representative of three similar experiments. B, HUVEC (gray bars) and C11 cell line (black bars) were treated with or without TNF (1 ng/ml) for 16 h in the presence of a vehicle, DMS (5 μM), S1P (5 μM), or PMA (100 ng/ml), respectively, cell viability was then assessed by an MTT assay. Data are the means ± S.D. from one experiment done in triplicate, which were repeated three times with similar results. C, after cells were treated as indicated, caspase-3/CPP32 was measured by Western blotting assay with anti-CPP32 antibodies. The activated form of the caspase appears as lower molecular mass (20 and 17 kDa) cleavage products.

Not only induced sphingomyelin hydrolysis but also activated ceramidase in a highly concentration-dependent manner in hepatocytes (38). Now we show that SphK can be activated by TNF independently of sphingomyelinase or ceramidase activity. The possibility of individual and independent activation of the enzymes in this metabolic pathway exists. This may be an important paradigm because bioactive sphingolipid metabolites have diverse effects, and the particular function of cytokine may be explained by a selectivity for the enzymes in question.

The mechanisms for TNF-promoted activation of SphK is not known although a novel signaling protein named FAN (factor associated with N-sphingomyelinase) has recently been described that can directly couple TNF receptor death domain to neutral sphingomyelinase activation (15). TRAF, a member of the adaptor protein family recruited to TNF receptors, has been shown to play a critical role in anti-apoptotic effect after TNF stimulation (10–13). Whether TNF-induced SphK activation needs the recruitment of TRAF to TNF receptor or vice versa is currently unknown. Another question raised from our study relates to the exact intracellular targets of S1P in protecting endothelial cells from death. Although they are not completely understood, several lines of evidence suggest the involvement of SphK activation in multiple anti-apoptotic signaling pathways. Previous studies from our laboratory and others have shown that S1P stimulated the extracellular signal-regulated kinase signaling pathway (19, 20) and activated the transcription factor, NF-κB (20). The activation of extracellular signal-regulated kinase and NF-κB have been considered as potent signals in protection against apoptosis in a variety of cell types (5, 9, 40). Activation of caspases is believed to be a central signal for mediating apoptotic cell death, and inhibition of caspases causes a resistance to apoptosis in response to multiple stimuli (30, 31). We found that co-treatment with S1P in C11 cell line protected TNF-induced CPP32 caspase activity. By contrast, in normal HUVEC that are resistant to TNF apoptotic effect, TNF failed to activate CPP32, whereas inhibition of SphK by DMS led the activation of CPP32 and sensitization to apoptosis upon TNF stimulation. These findings suggest an inhibitory effect of S1P on the caspase cascade. Recently, S1P has been reported to inhibit the activation of caspases that cleave poly(ADP-ribose) polymerase and lamins during Fas- and ceramide-mediated apoptosis in Jurkat T lymphocytes (33). Thus, the anti-apoptotic effect of S1P appears to be, at least partially, mediated through inhibition of caspase pathways.

In summary, TNF stimulation results in strikingly different cellular responses such as cell death or survival that were determined by the opposing signaling pathways simplified as Yin and Yang. For example, TNF induces the recruitment of FADD and TRAF (10), the activation of NF-κB and caspases (39), and we showed here the activation of SphK versus sphingomyelinase. The balance of such Yin and Yang could have profound effect to regulate the cytotoxic effect of TNF. A significant advance in understanding TNF self-control mechanism came recently with the finding that TNF activated NF-κB that provide cells with resistance against death through the induction of inhibitor-of-apoptosis proteins (3, 13, 40). This self-control mechanism could be important for designing a novel strategy to modulate TNF effect on cell death or survival in the systemic immune reaction and anti-cancer therapy.

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