

## Forum Review

# Redox Regulation of Apoptosis by Members of the TNF Superfamily

MEHDI SHAKIBAEI,<sup>1,2</sup> GUNDULA SCHULZE-TANZIL,<sup>2</sup> YASUNARI TAKADA,<sup>3</sup> and BHARAT B. AGGARWAL<sup>3</sup>

### ABSTRACT

**Tumor necrosis factor (TNF), fibroblast-associated cell surface (Fas) ligand, and TNF-related apoptosis-inducing ligand (TRAIL), all members of the TNF superfamily, are arguably the most potent inducers of cell death. These cytokines induce cell death through sequential recruitment by the death receptors TNFR1-associated death domain protein (TRADD), Fas-associated death domain protein (FADD), FADD-like interleukin-1 $\beta$ -converting enzyme (FLICE), and downstream caspases. Increasing evidence indicates that mitochondria play a critical role in cytokine receptor-mediated apoptosis. There is also now ample evidence that apoptosis induced by TNF and its family members is mediated through the production of reactive oxygen intermediates (also known as reactive oxygen species). Here we review the evidence linking reactive oxygen intermediates to cytokine-induced cell death mediated by TNF- $\alpha/\beta$ , Fas, TRAIL, TNF-like weak inducer of apoptosis (TWEAK), and vascular endothelial cell growth inhibitor (VEGI). *Antioxid. Redox Signal.* 7, 482–496.**

### INTRODUCTION

**T**UMOR NECROSIS FACTOR (TNF) was the first cytokine discovered to kill tumor cells by apoptosis (programmed cell death). After the ligand binds to its receptor, this receptor–ligand complex induces several different cellular responses, consisting of cellular proliferation, differentiation, survival, apoptosis, and activation of nuclear factor- $\kappa$ B (NF- $\kappa$ B) and mitogen-activated protein kinases (MAPK) (3, 74). There are 19 ligands and 29 receptors that belong to the TNF superfamily (Table 1), including TNF- $\alpha$  and TNF- $\beta$  (also known as lymphotoxin, LT), fibroblast-associated cell surface (Fas) ligand (FasL; also known as CD95L), TNF-like weak inducer of apoptosis (TWEAK), TNF-related apoptosis-inducing ligand (TRAIL), vascular endothelial cell-growth inhibitor (VEGI), and death receptor 6 (DR6).

Most of the TNF receptor superfamily members inducing apoptosis possess an intracellular death domain that recruits

several signaling proteins for the onset of apoptotic signaling cascades via mitochondria-independent mechanisms, finally leading to caspase (cysteiny l aspartic acid-protease) activation. Deletion of the death domain abolishes apoptosis (61, 62). It has been reported that TNF- $\alpha$ -mediated apoptosis can be induced by multiple signals, such as phospholipases, ceramide, p53, c-myc, protein kinases, serine proteases, caspases, reactive oxygen intermediates (ROI), and cytochrome *c* release (97, 130). Today more and more evidence indicates that an intensive cross talk exists between receptor-mediated and non-receptor-mediated, and between mitochondria-dependent, and mitochondria-independent, apoptotic pathways (109).

Members of the TNF superfamily are involved in a variety of physiological and pathological processes, including chronic heart failure, sepsis (117), arthritis (83), neovascularization, tumorigenesis, allograft rejection, meningitis, cancer-induced cachexia (9), and hepatocyte regeneration (2, 25).

<sup>1</sup>Institute of Anatomy, Ludwig-Maximilians-University Munich, Munich, Germany.

<sup>2</sup>Institute of Anatomy, Charité Medicine University Berlin, Campus Benjamin Franklin, Berlin, Germany.

<sup>3</sup>Cytokine Research Section, Department of Experimental Therapeutics, The University of Texas M.D. Anderson Cancer Center, Houston, TX.

TABLE 1. EXPRESSION OF THE LIGANDS (TNF- $\alpha$ , TNF- $\beta$ , CD95L, TWEAK, VEGI, TRAIL) OF THE TNF SUPERFAMILY AND THEIR RECEPTORS

Ligand	Cells	Receptor	Cells
TNF- $\alpha$	Macrophages	TNFR1	Most normal and transformed cells
	Natural killer cells	TNFR2	
	T- and B-lymphocytes		
TNF- $\beta$	Macrophages	TNFR1	Immune cells Endothelial cells
	Natural killer cells	TNFR2	
	T- and B-lymphocytes		
Fas	Activated thymocytes	FasL	Most normal and transformed cells Most normal and transformed cells
	Splenocytes		
	Cells of testis		
TRAIL	T-lymphocytes	DR4, DR5	Most normal and transformed cells
	Dendritic cells	DcR1, DcR2	
	Natural killer cells	OPG	
TWEAK	Monocytes	FN14	Endothelial cells Smooth muscle cells Immune cells Synoviocytes Fibroblasts
		TWEAKR2	
VEGI	Endothelial cells	DR3	Activated T-cells Endothelial cells
		DcR3	

### APOPTOSIS

Apoptosis (cell suicide/programmed cell death) is a normal and very important event in the life cycle of most cells in the organism. It is characterized by morphological and structural features involving mitochondrial swelling, release of cytochrome *c*, cytoplasmic membrane blebbing, chromatin condensation, caspase activation, DNA fragmentation, and cell fragmentation (127). Mitochondria play a crucial regulatory role early in the proceeding of apoptosis (44, 63). A commonly accepted marker of mitochondrial engagement during apoptosis is the release of cytochrome *c* from the mitochondrial intermembrane space to the cytosol. Once released in the cytosol, cytochrome *c* forms the “apoptosome” together with apoptosis protease-activating factor-1 (Apaf-1) and caspase-9. Apoptosome formation leads to the onset and irreversible progress of apoptosis (130). Mitochondrial cytochrome *c* release has been shown to be directly regulated by caspase-8 activation. Caspase-8 and -3 cleave the proapoptotic B-cell lymphoma 2 (Bcl-2) homology domain 3 (BH3)-interfering domain death agonist protein (Bid), which belongs to the Bcl-2 superfamily, and the cleavage product of Bid promotes cytochrome *c* release from mitochondria (12) (Fig. 1).

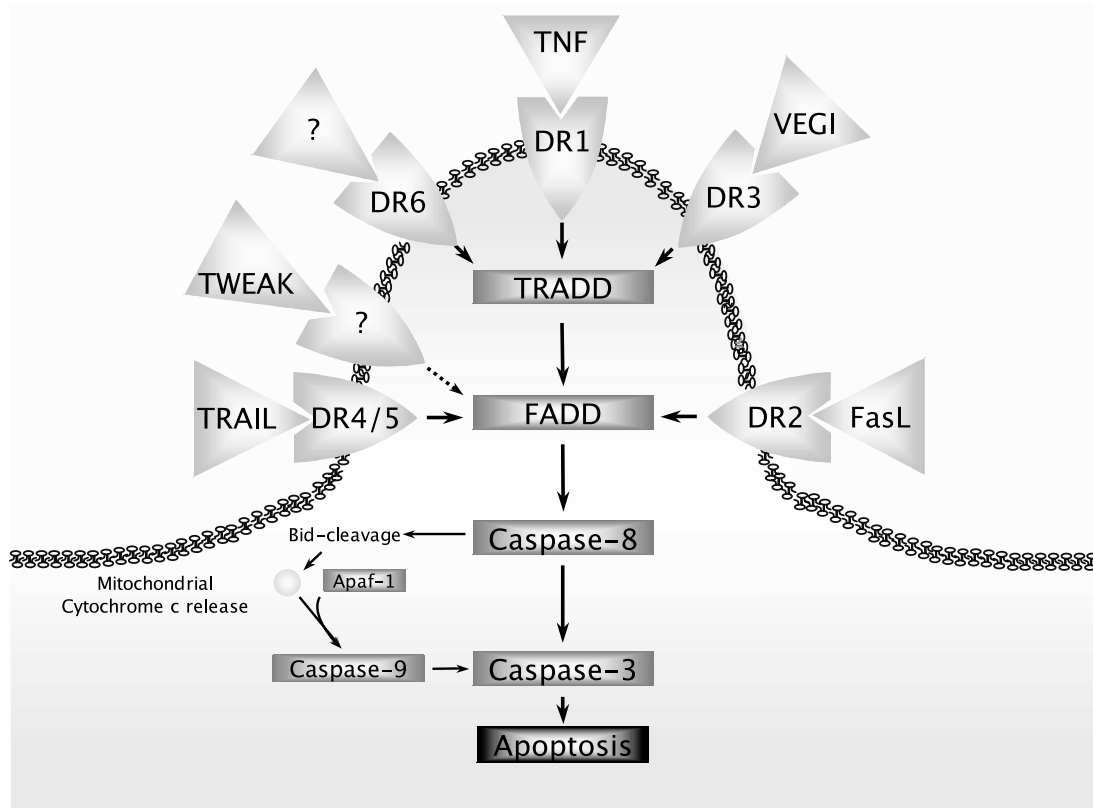
Apoptosis is mediated by the proteolytic actions of the cysteine proteases (caspases) (23). Several reports have demonstrated that members of the TNF superfamily are potent inducers of caspase-mediated apoptosis in a variety of cells and that apoptosis may be regulated by the activation of the ubiquitous central transcription factor NF- $\kappa$ B (13). Activation of NF- $\kappa$ B by TNF leads to a proliferative response, whereas when NF- $\kappa$ B is inhibited, TNF induces caspase-dependent cell apoptosis (5, 13, 128). Many of the nonapoptotic effects of TNF- $\alpha$ , such as up-regulation of proinflammatory genes, are regu-

lated by activator protein-1 (AP-1) and NF- $\kappa$ B. In many cell types such as cancer cells, NF- $\kappa$ B activation inhibits cell apoptosis, but it depends on the cell type (11).

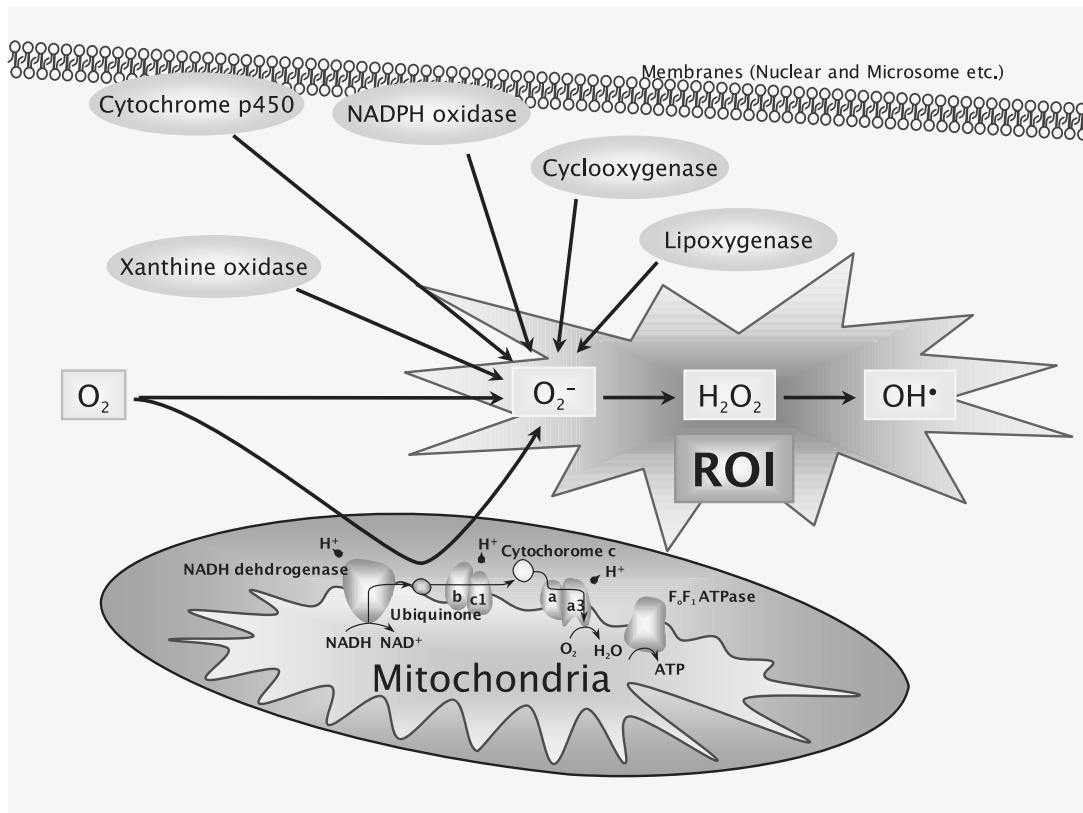
Recently, NF- $\kappa$ B has been found in the mitochondria, where it regulates their mitochondrial mRNA expression in response to cytokines (22). As TNF- $\alpha$  leads to activation of NF- $\kappa$ B, the function of mitochondrial NF- $\kappa$ B remains unclear. NF- $\kappa$ B also regulates the production of several proteins, such as the mitochondrial regulatory proteins Bcl-2, Bcl-x<sub>L</sub>, A1/Bfl-2, inhibitor of apoptosis protein (IAP), and TNF receptor-associated factor (TRAF), which inhibit the caspase cascade and block cytochrome *c* release from mitochondria (22, 24).

### ROI

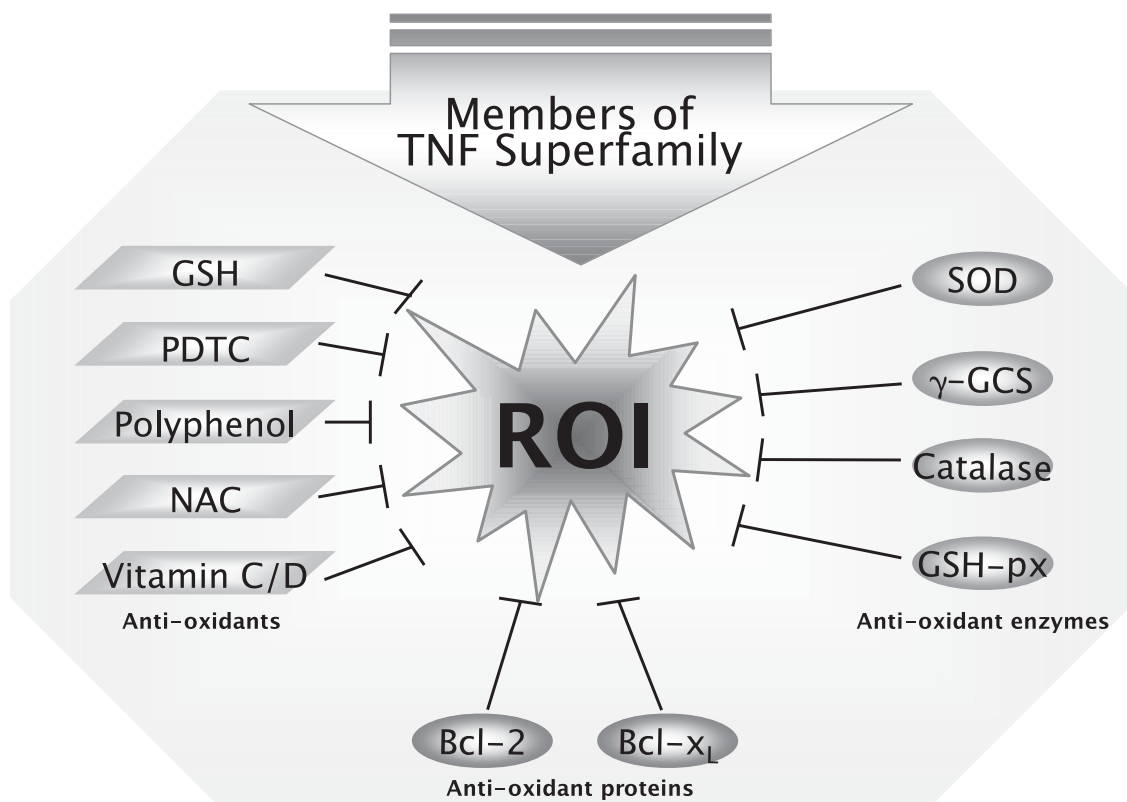
During endogenous metabolic reactions, aerobic cells produce ROI such as superoxide anion (O<sub>2</sub><sup>-</sup>), hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), hydroxyl radical (OH<sup>•</sup>), and organic peroxides as normal products of the biological reduction of molecular oxygen (32). The electron transfer to molecular oxygen occurs at the level of the respiratory chain, and the electron transport chains are located in membranes of the mitochondria, endoplasmic reticulum, the nucleus, and the cell, but mitochondria are the major source of cellular ROI (Fig. 2) (39, 41, 106). ROI regulate signal transduction in plant and animal cells (63), but an intracellular excess ROI caused by oxidative stress leads to cell death through lipid peroxidation, alteration of DNA, and various proteins. In contrast, a low amount of ROI is involved in the defense against microorganisms (108). The intracellular damaging effects of ROI are controlled by a system of enzymatic [e.g., superoxide dismutase (SOD), glutathione peroxidase (GSH-px), glutathione reductase, catalase] and nonen-



**FIG. 1. Apoptosis pathway induced by the members of the TNF superfamily.** Members of the TNF receptor superfamily interact with TRADD or FADD adaptor molecule and induce apoptosis through either caspase-8 activation or caspase-9 activation.



**FIG. 2. Major sources of ROI in the cell.**



**FIG. 3. Quenching of ROI by antioxidant enzymes, antioxidant proteins, and antioxidant chemicals.** The intracellular damaging effects of ROI are suppressed by a system of enzymatic and nonenzymatic antioxidants, which eliminate prooxidants and scavenge free radicals. The antioxidant proteins Bcl-2 and Bcl-xL are known to suppress ROI production through inhibition of the PTPC.

zymatic antioxidants [*e.g.*, glutathione (GSH), vitamins C and D], which eliminate prooxidants and scavenge free radicals (113) (Fig. 3).

### ROLE OF ROI IN CYTOKINE SIGNALING

A number of enzymes responsible for ROI synthesis have been identified (26), and a variety of stimuli, including mitogens, cytokines, and toxins, are able to activate the intracellular ROI (45, 97, 120). Several reports show that ROI release plays a role in TNF receptor-induced signaling and apoptosis (Table 2) (8, 35). Indeed, high levels of ROI have been shown in cells during apoptosis (119). Antioxidants inhibit apoptosis (75, 123), and an intracellular increase in oxidants activates apoptosis in cells (100). ROI production is an early feature of apoptosis (8). However, higher concentrations of ROI induce necrosis in cells (27, 55).

Small amounts of ROI function as intracellular messengers, mediating survival effects by, for example, increasing antiapoptotic factors (35). Taken together, oxidants and antioxidants have an important function in cellular physiology, and a delicate balance between them is needed for cellular homeostasis.

The exact TNF signal transduction pathways that activate ROI synthesis and the exact role of ROI-mediated proapoptotic and antiapoptotic signals are not fully known. Depend-

ing on cell type, several antiapoptotic effects mediated by ROI in response to TNF-superfamily ligands seem to involve NF- $\kappa$ B activation (Figs. 1 and 4) (35). Activation of caspase-3 and -8 and serine proteases by ROI may contribute to apoptosis (35, 64, 71, 132). TNF-induced activation of NF- $\kappa$ B requires the mitochondrial electron transport system, because the presence of rotenone (the protein complex I inhibitor) blocks NF- $\kappa$ B activation (106).

### ROLE OF ROI IN CASPASE ACTIVATION

Caspases are a specialized family of proteases that execute apoptosis by destroying structural and functional cell proteins. The members of the caspase family contain a cysteine-rich residue at the active site and cleave targeted proteins after aspartic acid. They are synthesized as proenzymes and activated by removal of their NH<sub>2</sub>-terminal prodomain. Caspase-8 and -10 are crucial proximal caspases in the caspase cascades. Caspases play a role in death receptor-mediated apoptosis because these caspases are recruited by Fas-associated death domain (FADD) (1). Caspase-3 is an important downstream executioner caspase that cleaves a wide variety of substrates important for the onset of apoptosis, such as antiapoptotic proteins of the Bcl-2 superfamily (Bcl-2, Bid), inhibitor of caspase-activated deoxyribonuclease (ICAD), and NF- $\kappa$ B (10).

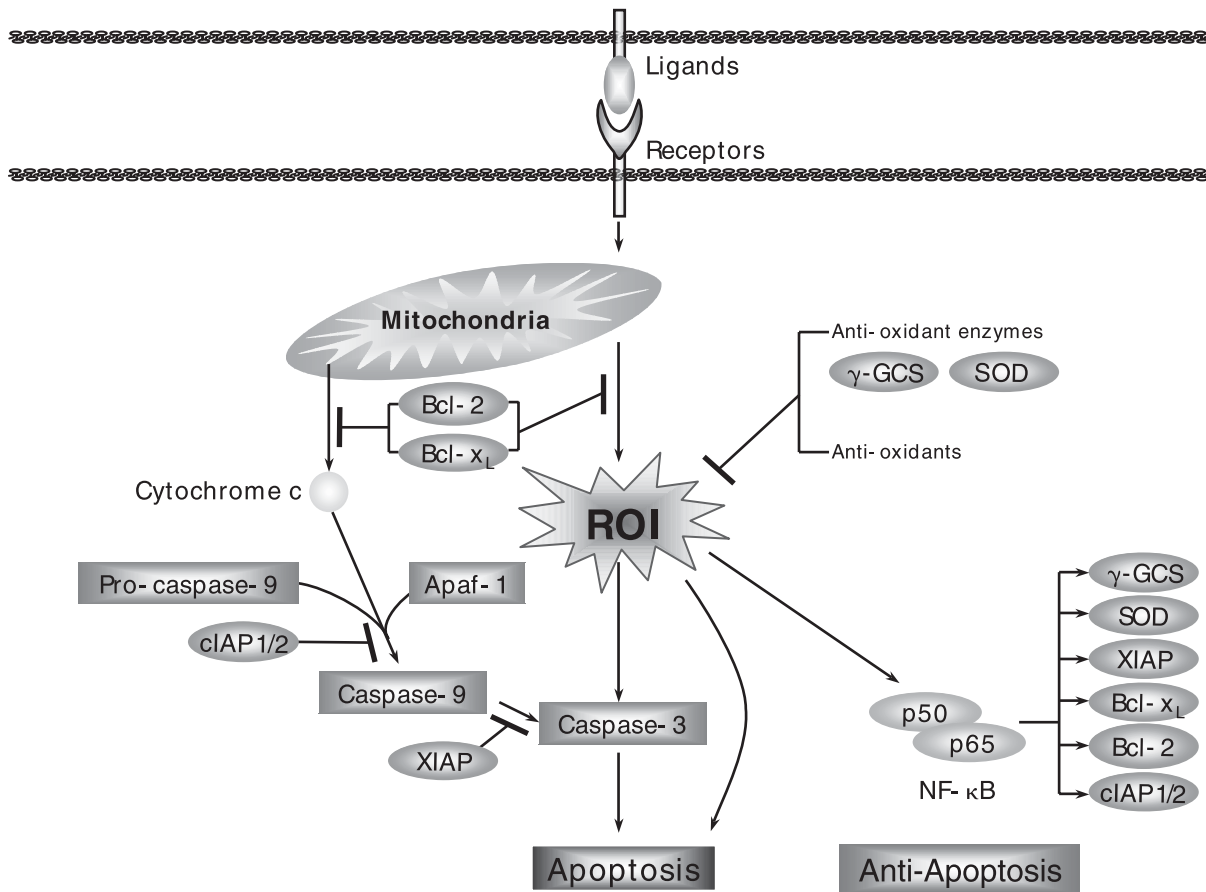
TABLE 2. ROLE OF ROI IN TNF SUPERFAMILY-MEDIATED CELL APOPTOSIS

<i>Ligands</i>	<i>References</i>
<b><i>TNF-<math>\alpha</math></i></b>	
TNF- $\alpha$ potentiates ROI by activating p38 MAPK	31
TNF- $\alpha$ increases the production of ROI	121
TNF- $\alpha$ -induced necrosis by caspase-regulated ROI production	71
PAF- $\alpha$ -induced NF- $\kappa$ B mediated by ROI	21
Anethole inhibits TNF- $\alpha$ -induced ROI	16
ROI induces apoptosis	64
ROI-dependent NF- $\kappa$ B-mediated transcription of cytokines	86
Coronary smooth muscle cells are potential source of ROI	70
TNF- $\alpha$ causes hypertrophy in cardiac myocytes via ROI generation	84
TNF- $\alpha$ increased ROI release in macrophages	99
Combination of TNF- $\alpha$ and heat shock induced ROI expression and led to apoptosis	124
Mitochondrial ROI induction by TNF	40
TNF- $\alpha$ induces mitochondrial ROI and cytotoxicity	39
DNA damage induced by TNF- $\alpha$ mediated by ROI	110
Involvement of ROI in COX-2 expression by TNF- $\alpha$	29
ROI mediate TNF- $\alpha$ gene expression	95
<b><i>TNF-<math>\beta</math></i></b>	
Role of ROI release in TNF- $\beta$ -mediated apoptosis has not been investigated	
TNF- $\beta$ -induced cytotoxicity does not depend on ROI	94
<b><i>Fas</i></b>	
Fas-induced apoptosis does not depend on ROI	59
Fas-induced apoptosis leads to ROI release, disruption of $\Delta\psi_m$	8
Fas-induced pathway of cell suicide	122
Fas-induced cell death is mediated by Ras-regulated O <sub>2</sub> <sup>-</sup> synthesis	46
Mitochondrial transmembrane potential and ROI in Fas signaling	90
ROI and antiapoptotic factors	66
ROI is important in Fas-mediated apoptosis	4
<b><i>TRAIL</i></b>	
No information is available about a possible cross talk between TRAIL and ROI	
Mitochondrial amplification loop for TRAIL signaling	115
Loss of mitochondrial potential and cytochrome <i>c</i> release in response	58
<b><i>TWEAK</i></b>	
Role of ROI release in TWEAK-mediated apoptosis has not been investigated	
Caspase inhibition sensitizes for TWEAK-induced necrosis by ROI	85
<b><i>VEGI/DR6</i></b>	
Interactions of ROI have not been investigated	

During TNF-induced apoptosis, a number of intracellular proteins are activated (97), in a pathway that is mediated by ROI (Fig. 4). Reports have shown that ROI mediate both proapoptotic and prosurvival signals (Fig. 4) (35). ROI-mediated apoptosis has been shown in hepatocytes to be caspase-dependent or -independent, depending on the particular inducing ROI (64). Such ROI as H<sub>2</sub>O<sub>2</sub> and superoxide were able to activate upstream (caspase-2 and -8) and downstream (caspase-3 and -7) caspases in hepatocytes (64). Furthermore, ROI play an important role in the TNF-signaling pathway as an upstream target in TNF-induced apoptosis and caspase activation. However, overexpression of such antioxidant enzymes as  $\gamma$ -glutamylcysteine synthetase ( $\gamma$ -GCS) and SOD inhibits TNF-induced cytotoxicity and caspase activation (76, 77), and various ROI quenchers block TNF-induced caspase activation (111) (Fig. 3).

## ROLE OF ROI IN CYTOCHROME C RELEASE

The production of ROI and oxidative stress conditions are increased by a variety of stimuli, *e.g.*, drugs, ionizing radiation, and binding of cytokines to the cell-surface receptors (15). Several reports have revealed that the mitochondrial electron transport system and the redox potential of the cells play a key role in inducing TNF cytotoxicity by the formation of ROI (Fig. 3) (30, 37, 60, 68, 82, 87, 91, 92, 105, 110). It has been reported that TNF- $\alpha$  changes the redox potential of the cells (37, 92, 103) and that the altered redox potential leads to activation of transcription factors such as NF- $\kappa$ B to induce downstream gene expressions. This signaling mechanism is inhibited by the antioxidant *N*-acetylcysteine (NAC) and metal chelators (103, 104, 116) (Fig. 3).



**FIG. 4. ROI mediates both pro- and antiapoptotic signaling.** The proapoptotic effects of ROI are mediated through caspase activation, and antiapoptotic effects are mediated via NF-κB activation.

Mitochondria are the major source of cellular ROI involved in TNF-α-induced apoptosis (39, 106). Treatment of cells with TNF-α alters mitochondrial membrane permeability, inhibits respiratory chain complex I, induces mitochondrial swelling and clustering, and leads to cytochrome *c* release. Cytochrome *c* activates caspases that kill the cell (17, 39, 89, 97, 106). Chandel *et al.* have reported that TRAF2 directs signaling generated by TNF to the electron transport mechanism in mitochondria and then to the production of ROI (17). However, the expression of manganese SOD, radical scavengers, and inhibitors of the mitochondrial electron transport chain protect the cells and provide evidence for the direct involvement of ROI in TNF signaling and cytotoxicity (105, 106, 126). The exact TNF signal transduction pathways activated by ROI synthesis and the exact role of ROI-mediated proapoptotic and antiapoptotic signals are not fully understood. The distinct role of ROI in the TNF signaling pathway that leads to apoptosis is still not elucidated and is the topic of this review.

### ROI AFFECTS MITOCHONDRIAL MEMBRANE POTENTIAL

The mitochondrial membrane potential ( $\Delta\psi_m$ ) is dependent on the electron transport chain transferring electrons

from NADH to molecular oxygen and a proton transport mediated by the  $F_0F_1$ -ATPase complex (114). The energy stored in the electrochemical gradient is used by  $F_0F_1$ -ATPase to convert ADP to ATP during oxidative phosphorylation. Therefore,  $\Delta\psi_m$  plays an important role for energy (ATP) production, but also in cell signaling. Many proapoptotic stimuli can affect the  $\Delta\psi_m$ , such as Fas,  $H_2O_2$ , p53, TNF, and staurosporine (8, 47, 72, 96, 101). These agents cause an elevated membrane potential and release of ROI. Elevation of  $\Delta\psi_m$  is independent of activation of caspases and happens early during apoptosis (8, 72). Hyperpolarization of the mitochondrial inner membrane followed by increased ROI production could be shown in activated T-cells of systemic lupus erythematosus patients (36).

At the inner-outer membrane contact sites, mitochondrial pores and megachannels can be formed in response to apoptotic stimuli. This permeability transition pore complex (PTPC) consists of different transporters, channels, and outer membrane proteins. The PTPCs allow diffusion of low-molecular-mass compounds such as cytochrome *c* and lead to disruption of the mitochondrial membrane. Loss of membrane potential appears to be a point of no return in the effector phase of apoptosis (90). Inhibition of these dynamic multiprotein pore complexes can prevent loss of  $\Delta\psi_m$ . PTPCs induce ROI release (79). Furthermore, a specific inhibitor of PTPCs pre-

vents translocation of NF- $\kappa$ B (79). Mitochondrial permeability transition that leads to alteration of the cellular redox state seems to be a central coordinator of diverse ROI-dependent signaling pathways and plays a pivotal role in the induction phase of apoptosis (63).

Bcl-2 proteins were suggested to inhibit apoptosis by direct regulation of the PTPC (79) (Fig. 3). Bcl-2 is anchored in the mitochondrial outer membrane and colocalizes with the PTPC at the contact sites between the inner and outer mitochondrial membranes (63).

## ROLE OF ROI IN NF- $\kappa$ B ACTIVATION

In the inactive state, NF- $\kappa$ B is present in the cytoplasm of cells in a complex consisting of two subunits and an additional inhibitory subunit I $\kappa$ B $\alpha$ . Five different subunits exist, *i.e.*, c-Rel, RelA, RelB, p50/p105, and p52/p100, which can form homo- or heterodimers in various combinations. During activation, the inhibitory subunit I $\kappa$ B $\alpha$  is phosphorylated by I $\kappa$ B kinase (IKK) and subsequently degraded. Once released, subunits of activated NF- $\kappa$ B translocate to the nucleus to bind to NF- $\kappa$ B recognition sites in the promoters of various genes, thus regulating gene expression (109).

The NF- $\kappa$ B plays a controversial role in TNF superfamily-induced signaling pathways (109). The TNF superfamily is a potent inducer of NF- $\kappa$ B; once activated, NF- $\kappa$ B inhibits TNF-stimulated apoptosis (7, 38). It regulates not only antiapoptotic signals, but also proapoptotic signaling by the regulation of death receptors (1).

ROI have been strongly implicated in activation of NF- $\kappa$ B, AP-1, c-Jun N-terminal kinase (JNK), mitogen-activated protein kinase kinase (MEK), and caspase activation in response to cytokines such as interleukin-1 $\beta$  (IL-1 $\beta$ ) and TNF- $\alpha$  (21, 70, 103, 111). ROI seem to be common mediators of the TNF gene-regulatory signaling pathways (106). But the exact mechanism for ROI-induced NF- $\kappa$ B activation remains unclear (86, 103). We do know that ROI mediate antiapoptotic events via NF- $\kappa$ B activation leading to transcription of antiapoptotic genes, including extracellular growth factor and IL-1 (34, 35, 69). In contrast to the known protective effect of NF- $\kappa$ B activation in TNF- $\alpha$ -induced hepatocyte apoptosis, NF- $\kappa$ B was proved to promote hepatocellular death from ROI in these cells (64).

Therefore, in the same cell type, NF- $\kappa$ B can promote or inhibit apoptosis depending on the apoptotic stimulus or ROI species (64, 111). The mechanism by which NF- $\kappa$ B promotes ROI-induced death is unknown. It has been reported that ROI are able to activate either NF- $\kappa$ B or caspases in ROI-mediated apoptosis (47, 55, 80). However, overexpression of the antioxidant enzymes such as  $\gamma$ -GCS can antagonize ROI and inhibit TNF-induced cytotoxicity and activation of caspases, NF- $\kappa$ B, AP-1, JNK, and MEK (77). Others have shown that some antioxidants, which inhibit NF- $\kappa$ B, can activate AP-1 (33, 102), demonstrating a common upstream pathway for NF- $\kappa$ B and AP-1, but different downstream pathways. Moreover, TNF-induced activation of NF- $\kappa$ B requires the mitochondrial electron transport system, because the presence of rotenone (the protein complex I of respiratory chain inhibitor)

blocks NF- $\kappa$ B activation (106). In contrast, Higuchi *et al.* reported that both ROI-independent and -dependent pathways are possible for activation of NF- $\kappa$ B (56). Hayakawa *et al.* recently presented evidence that ROI do not mediate NF- $\kappa$ B activation (52). They showed that both NAC and pyrrolidine dithiocarbamate (PDTTC) inhibit NF- $\kappa$ B activation independently of antioxidative function. NAC selectively blocked TNF-induced signaling by lowering the affinity of receptor to TNF. PDTTC inhibited the I $\kappa$ B $\alpha$ -ubiquitin ligase activity in the cell-free system where extracellular stimuli-regulated reactive oxygen species production did not occur. Furthermore, they showed that endogenous reactive oxygen species produced through Rac/NADPH oxidase did not mediate NF- $\kappa$ B signaling, but instead lowered the magnitude of its activation.

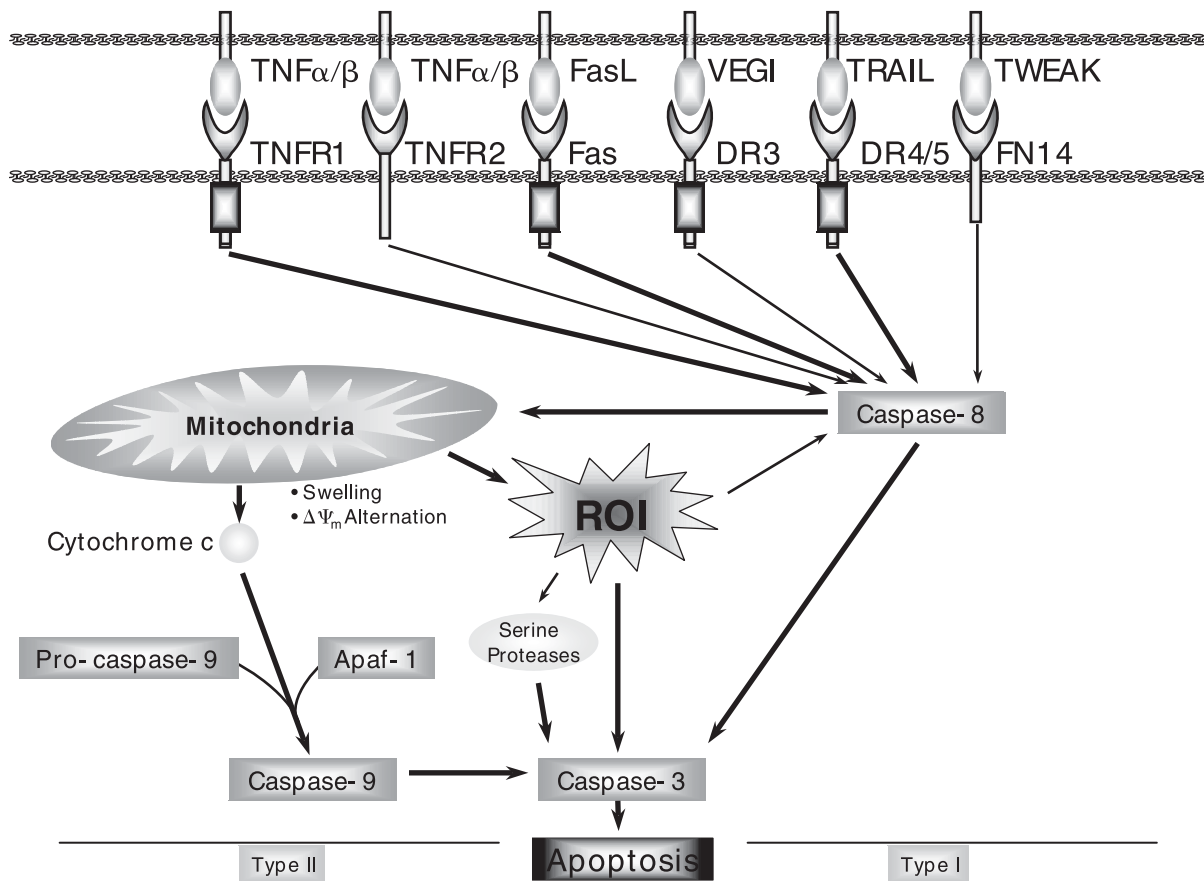
## ROLE OF ROI IN EFFECTS MEDIATED BY ANTIAPOPTOTIC PROTEINS

It has been proposed that ROI mediate both pro- and antiapoptotic signaling (Fig. 3) (36). As mentioned above, ROI lead to activation of NF- $\kappa$ B (21, 70, 103). One group of target genes regulated by NF- $\kappa$ B are members of the Bcl-2 superfamily, such as Bcl-2 and Bcl-x<sub>L</sub> (35). Members of the Bcl-2 family are known to affect the redox status of cells (78). Antiapoptotic proteins of the Bcl-2 family, such as Bcl-2, Bcl-2-associated gene product-1 (BAG-1), and Bcl-x<sub>L</sub>, reside in the outer mitochondrial membrane. The ratio between pro- and antiapoptotic members of the Bcl-2 family has a major influence on the onset of apoptosis. This location is also the major source for ROI release. The Bcl-2 proteins colocalize with PTPC of mitochondria (63). Despite the close proximity of ROI and Bcl-2 family members, a direct interaction between them has not been shown at the moment. It has been demonstrated that treatment of cells with oxidants leads to apoptosis that can be inhibited by Bcl-2 overexpression (57, 65). Furthermore, Bcl-x<sub>L</sub> suppresses TNF-mediated apoptosis and activation of NF- $\kappa$ B, AP-1, and JNK (78). Therefore, Bcl-2 somehow seems to inhibit cell death via protection from oxidative stress, but the particular signaling pathway remains to be elucidated (Fig. 4).

## ROLE OF ROI IN TNF- $\alpha$ -INDUCED APOPTOSIS

TNF- $\alpha$  interacts with TNF- $\alpha$  receptor 1 and 2 (TNFR1 and TNFR2) and participates in a variety of cellular responses, including antiviral activity, transcription factor activation, immune response regulation, cytotoxicity, and apoptosis (Fig. 5). Many of the proinflammatory or antiapoptotic effects of TNF- $\alpha$  signaling are mediated by activation of NF- $\kappa$ B (35). Recently, a direct effect of TNF- $\alpha$  signaling on the mitochondrial electron transport mechanism resulting in ROI release from mitochondria has been shown (17, 39). A number of reports have shown that ROI is involved in TNF-induced signaling in different cell types (54, 106, 111, 112).

TNF-induced mitochondrial superoxide production leads to cell cytotoxicity, and this effect could be inhibited by ROI



**FIG. 5. Members of the TNF superfamily induce apoptosis via receptor-dependent (type I) and mitochondria-dependent (type II) pathways.**

scavengers, such as butylated hydroxyanisole (39, 111). A potent antioxidant, glutathione inhibits TNF-induced apoptosis by blocking neutral sphingomyelinase in human breast cancer cells, and an increase in glutathione levels leads to inhibition of the activation of NF-κB (30, 39, 68, 73, 82, 87, 103, 110, 116). A decrease in glutathione levels leads to activation of TNF-induced apoptosis (54, 87, 107). Furthermore, a mitochondrial respiration inhibitor blocks TNF-induced cytotoxicity and differentiation (54). ROI serve as second messengers in cell signaling, and ROI scavengers can effectively block TNF-induced cytotoxicity, underlining the crucial role of ROI in cytotoxicity (39, 106). Formation of ROI was found to lead to DNA damage after TNF treatment (110).

Some other reports have focused on ROI as a common upstream signaling target in TNF-α-induced apoptosis (35). On the other hand, ROI have also been implicated in antiapoptotic signaling in response to TNF-α. TNF-α induces activation of NF-κB whose activation blocks TNF-induced apoptosis (35). Furthermore, ROI were shown to mediate TNF-induced gene transcription via NF-κB and AP-1 activation (35). Therefore, ROI appear to play a role in both pro- and antiapoptotic signaling in response to TNF-α. ROI are also implicated in the induction of TNF-α mRNA in response to UVB radiation, thus enhancing TNF-α signaling (95). Newman *et al.* found that ROI formation in smooth coronary muscle cells was accom-

panied by release of TNF-α (86). Gossart *et al.* found that ROI regulate TNF-α production in alveolar macrophages (42). NF-κB mediates the transcription of both antiapoptotic and proapoptotic genes (35). It has been shown that anethole, a constituent of anise, camphor, and fennel, through suppression of ROI generation inhibits NF-κB activation. Anethole suppressed TNF-induced inflammatory effects mediated by AP-1, JNK, and MEK and suppressed TNF-induced apoptosis (16), emphasizing that activation of NF-κB by ROI also mediates proapoptotic effects. It is not clear which effects disturb the balance between pro- and antiapoptotic effects induced by ROI via NF-κB (Fig. 4). The net effect may depend on cell type, the microenvironment of cells such as culture conditions, and other interacting factors.

### ROLE OF ROI IN TNF-β-INDUCED APOPTOSIS

TNF-β binds to TNFR1 and TNFR2, but TNFR2 lacks a death domain (10). As a result, TNFR2 does not usually mediate apoptosis, but overexpression of TNFR2 leads to apoptosis (50). The apoptotic signaling pathway of TNFR2 is not understood at the moment (10). Powell *et al.* found that TNF-β-induced cytotoxicity could not be inhibited by the oxygen



radical scavenger glutathione, and therefore concluded that this cytotoxicity does not depend on oxygen radicals in human ovarian and cervical carcinoma cells (94). The direct effect of TNFR2 signaling on ROI release remains to be investigated.

### ROLE OF ROI IN FAS-INDUCED APOPTOSIS

The cell-surface receptor Fas (also known as DR2 or CD95/Apo-1 receptor) contains a death domain that mediates cell apoptosis via interaction with FADD. The Fas-FADD complex associates further with caspase-8 [FADD-like IL-1 $\beta$ -converting enzyme (FLICE)] and caspase-3 activation (1, 10). An alternative mitochondria-mediated pathway has also been described for Fas signaling, consisting of cleavage of Bid protein by caspase-8 (10).

ROI plays a crucial role in mediating Fas-dependent apoptosis (4, 122) because the Fas-induced apoptosis was completely abolished by antioxidants such as NAC and glutathione (46, 122). The Fas receptor activates ras in response to ligand binding, and activated ras leads to generation of ROI (46). Furthermore, ras was shown to be activated via sphingomyelinases or ceramide in lymphocytes. Disruption of mitochondrial transmembrane potential ( $\Delta\psi_m$ ) and ROI release occurs early in Fas-mediated apoptosis (8). The initial increase in ROI is followed by elevation of  $\Delta\psi_m$ , externalization of phosphatidylserine, and later disruption of  $\Delta\psi_m$ , which together mediate apoptosis. Cell death, externalization of phosphatidylserine, and disruption of  $\Delta\psi_m$  could be inhibited by caspase inhibitors, suggesting that these events depend on caspase activation. But elevated ROI and elevated  $\Delta\psi_m$  levels persisted (8). Taken together, the balance between mitochondrial ROI, reducing agents, and other factors regulates the susceptibility to Fas-induced apoptosis. Protein phosphatase 2, for example, has been shown to be an essential factor for survival and growth of cells via regulation of intracellular ROI and antiapoptotic factors in Fas-mediated apoptosis (66). There are other reports, however, that show lack of requirements of ROI in Fas-mediated apoptosis (59). The latter studies were based on the use of antioxidants.

### ROLE OF ROI IN TWEAK-INDUCED APOPTOSIS

TWEAK is the ligand that binds to fibroblast growth factor inducible-14 receptor [FN14; also known as TWEAK receptor (TWEAKR)] and was initially described in 1997 (19). TWEAK is a cell surface-associated type II transmembrane protein of the TNF superfamily, but a smaller, biological active form of TWEAK also exists that can be shed in the extracellular milieu (125). TWEAK is implicated in apoptosis, proliferation, migration, inflammation, and angiogenesis (93, 98). It has proinflammatory activity by up-regulating proteins such as prostaglandin E2, matrix metalloproteinase-1, IL-6, cell adhesion molecule (ICAM-1), and E-selectin, and induces secretion of some chemokines such as IL-8 (20, 49). TWEAK

signaling has been shown in monocytes, macrophages, smooth muscle cells, fibroblasts, synoviocytes, and endothelial cells. Recently, signaling by a second TWEAK (TWEAKR2) receptor has been reported (93).

TWEAK binding to TWEAKR2 activates NF- $\kappa$ B, JNK, and MEK signaling cascade, as is the case for FN14. In contrast to FN14, the MAPK activity was stronger when binding to TWEAKR2 (93). Depending on cell type, TWEAK induces multiple pathways of cell death, including caspase-dependent apoptosis, cathepsin B-dependent necrosis, and endogenous TNF- $\alpha$ -dependent cell death. These multiple death pathways seem to be solely mediated by FN14 even though this receptor lacks an intracellular death domain (85). Nakayama's group was able to show in FN14 transfectants that the pan-caspase inhibitor sensitized the transfectants to TWEAK-induced death by necrosis via ROI and cathepsin B-dependent pathways (85). Therefore, one can suggest that some of these death pathways, *e.g.*, by ROI and cathepsin B, are caspase-independent. In contrast, TWEAK-induced apoptosis was associated with caspase-8 and -3 activation (85). Furthermore, TWEAK has been identified as an effective inducer of constitutive NF- $\kappa$ B activation (98). Unlike other ligands of the TNF family, TWEAK leads to prolonged NF- $\kappa$ B (8–24 h) activation. TWEAK-induced NF- $\kappa$ B activation depends on the adaptor molecules TRAF2 and TRAF5 (48, 98).

Upon TWEAK binding, the cytoplasmic tail of FN14 interacts and associates with the adaptor molecules TRAF1, 2, 3, and 5 for activation of the NF- $\kappa$ B signaling pathway (14, 48). The question arises whether ROI play a role in TWEAK-induced death signaling because it is known that ROI are downstream products of TRAF-mediated signal transduction (17).

### ROLE OF ROI IN TRAIL-INDUCED APOPTOSIS

TRAIL is the ligand that binds to the death receptors DR4 and DR5, but also to the decoy receptors DcR1 and DcR2, as well as osteoprotegerin (OPG) (1). Decoy receptors allow ligand binding without transducing a signal, thus regulating TNF signaling. DcR1 and DcR2 lack an intracellular death domain or contain a nonfunctional death domain. DR4 and DR5 lead to apoptosis by activating TNFR1-associated death domain protein (TRADD) followed by activation of caspase-8 and -3 (1). Caspase-8 can cleave proapoptotic Bid, and the cleaved Bid leads to release of mitochondrial cytochrome *c*. TRAIL-induced apoptosis is negatively regulated by several cellular factors (1). NF- $\kappa$ B regulates TRAIL expression, and like all members of the TNF superfamily, TRAIL is able to activate NF- $\kappa$ B (1).

TRAIL is considered to induce apoptosis in a variety of cancer cells, but not in normal cells, so it is under investigation as a potentially powerful cancer therapeutic (6, 58). The susceptibility to TRAIL-mediated apoptosis depends further on the differentiation state of cells. Blocking of the function of NF- $\kappa$ B has been shown to lead to enhanced susceptibility of cells for TRAIL-induced apoptosis (28). The apoptosis in differentiating cells induced by TRAIL was suggested to be independent from mitochondrial pathways (53). In combina-

tion with chemotherapeutics, a synergistic effect on mitochondria (loss of mitochondrial potential, release of cytochrome *c*) has been shown (58, 81). In contrast, Soderstrom *et al.* described a mitochondrial amplification loop for TRAIL signaling evident by mitochondrial depolarization and cytochrome *c* release in response to TRAIL stimulation (115). The mitochondrial effects of TRAIL could be inhibited by MAPK signaling (115). Taken together, the evidence shows that TRAIL-induced apoptosis depends on mitochondria-dependent and -independent signaling pathways (118), but at the moment no information is available about the role of ROI in TRAIL-mediated apoptosis.

### ROLE OF ROI IN VEGI-INDUCED APOPTOSIS

VEGI belongs to the TNF superfamily and binds to the DR3 and DcR3 (10). It inhibits proliferation and neovascularization by endothelial cells (131) and seems so far to be endothelial cell-specific. VEGI leads to growth arrest and programmed cell death in proliferating cells, but not in nonproliferating cells as shown by caspase-3 activation and annexin V labeling (129). The expression of VEGI is regulated by inflammatory cytokines such as TNF- $\alpha$  (18).

DR3, the receptor for VEGI, interacts with TRADD and forms a complex with caspase-8. VEGI mechanisms are similar to those of other members of the TNF superfamily such as NF- $\kappa$ B (51). VEGI also activates JNK (51). Nothing is known at present about the role of ROI in VEGI-induced apoptosis, however.

### ROLE OF ROI IN DR6-INDUCED APOPTOSIS

DR6 is expressed in most human tissues. It contains a cytoplasmic death domain and four extracellular cysteine-rich motifs. DR6 interacts with TRADD (10). TNF induces the expression of DR6 by activation of NF- $\kappa$ B (67). Ectopic expression of DR6 in mammalian cells has been shown to induce apoptosis and to activate NF- $\kappa$ B and JNK (88). The ligand for DR6 is unknown at the moment, as is the role of ROI in DR6-induced apoptosis.

### CONCLUSIONS

The regulatory role of mitochondria in apoptosis has been underestimated for a long time. Formerly, two separate death pathways had been hypothesized: death receptor-mediated apoptosis and apoptosis induced by mitochondrial alterations. But it has become more and more evident that separate pathways do not exist. In the last few years, several death receptor-mediated pathways were proven to cross-talk intensively with mitochondrial signaling pathways. Mitochondria play a crucial regulating and enhancing role in death and other signaling events.

The precise redox regulation of cells orchestrated by mitochondrial enzyme complexes contributes significantly to cell survival. Alterations in the redox potential of cells can change signaling leading to apoptosis. The balance between ROI and antioxidants and between pro- and antiapoptotic factors commits the cell to survival or apoptosis or necrosis. The converse roles of ROI and NF- $\kappa$ B in cell-death and -survival signaling depend on multiple interacting pathways that involve redox regulation of mitochondria. Therefore, ROI seem to be not only by-products of metabolic reactions of the organism, but also important signaling messengers that represent a target upstream of NF- $\kappa$ B activation. ROI may represent an early and very common signaling target upstream of NF- $\kappa$ B activation at a cross-point of multiple interacting pathways. Much work remains to be done to elucidate further its precise role in the signaling cascades of TNF receptors.

### ACKNOWLEDGMENTS

This work was supported partially by the Clayton Foundation for Research (to B.B.A.), Department of Defense U.S. Army Breast Cancer Research Program grant BC010610 (to B.B.A.), a PO1 grant (CA91844) from the National Institutes of Health on Lung Cancer Chemoprevention (to B.B.A.), and a P50 Head and Neck SPORE grant from the National Institutes of Health (to B.B.A.).

### ABBREVIATIONS

AP-1, activator protein-1; Apaf-1, apoptosis protease-activating factor-1; Bcl-2, B-cell lymphoma 2; Bid, BH3-interacting domain death agonist; caspase, cysteinyl aspartic acid-protease; DcR, decoy receptor; DR, death receptor; FADD, Fas-associated death domain protein; Fas, fibroblast-associated cell surface; FasL, Fas ligand; FLICE, FADD-like interleukin-1 $\beta$ -converting enzyme; FN14, fibroblast growth factor inducible-14 receptor;  $\gamma$ -GCS,  $\gamma$ -glutamylcysteine synthetase; H<sub>2</sub>O<sub>2</sub>, hydrogen peroxide; IAP, inhibitor of apoptosis protein; IL, interleukin; JNK, c-Jun N-terminal kinase; MAPK, mitogen-activated protein kinase; MEK, mitogen-activated protein kinase kinase; NAC, *N*-acetylcysteine; NF- $\kappa$ B, nuclear factor- $\kappa$ B; OPG, osteoprotegerin; PDTC, pyrrolidine dithiocarbamate; PTPC, permeability transition pore complex; ROI, reactive oxygen intermediates; SOD, superoxide dismutase; TNF, tumor necrosis factor; TNFR, TNF receptor; TRADD, TNFR1-associated death domain protein; TRAF, TNF receptor-associated factor; TRAIL, TNF-related apoptosis-inducing ligand; TWEAK, TNF-like weak inducer of apoptosis; TWEAKR, TWEAK receptor; VEGI, vascular endothelial cell growth inhibitor;  $\Delta\psi_m$ , mitochondrial membrane potential.

### REFERENCES

1. Aggarwal BB. Signalling pathways of the TNF superfamily: a double-edged sword. *Nat Rev Immunol* 3: 745–756, 2003.

2. Aggarwal BB and Vilcek J. *Tumor Necrosis Factor: Structure, Function and Mechanism of Action*. New York: Marcel Dekker, 1992.
3. Arch RH and Thompson CB. Lymphocyte survival—the struggle against death. *Annu Rev Cell Dev Biol* 15: 113–140, 1999.
4. Aronis A, Melendez JA, Golan O, Shilo S, Dieter N, and Tirosh O. Potentiation of Fas-mediated apoptosis by attenuated production of mitochondria-derived reactive oxygen species. *Cell Death Differ* 10: 335–344, 2003.
5. Ashkenazi A and Dixit VM. Death receptors: signaling and modulation. *Science* 281: 1305–1308, 1998.
6. Baetu TM and Hiscott J. On the TRAIL to apoptosis. *Cytokine Growth Factor Rev* 13: 199–207, 2002.
7. Bakker TR, Reed D, Renno T, and Jongeneel CV. Efficient adenoviral transfer of NF-kappaB inhibitor sensitizes melanoma to tumor necrosis factor-mediated apoptosis. *Int J Cancer* 80: 320–323, 1999.
8. Banki K, Hutter E, Gonchoroff NJ, and Perl A. Elevation of mitochondrial transmembrane potential and reactive oxygen intermediate levels are early events and occur independently from activation of caspases in Fas signaling. *J Immunol* 162: 1466–1479, 1999.
9. Beutler B. Cytokines and cancer cachexia. *Hosp Pract (Off Ed)* 28: 45–52, 1993.
10. Bhardwaj A and Aggarwal BB. Receptor-mediated choreography of life and death. *J Clin Immunol* 23: 317–332, 2003.
11. Bharti AC and Aggarwal BB. Nuclear factor-kappa B and cancer: its role in prevention and therapy. *Biochem Pharmacol* 64: 883–888, 2002.
12. Bossy-Wetzell E and Green DR. Caspases induce cytochrome *c* release from mitochondria by activating cytosolic factors. *J Biol Chem* 274: 17484–17490, 1999.
13. Bradham CA, Qian T, Streetz K, Trautwein C, Brenner DA, and Lemasters JJ. The mitochondrial permeability transition is required for tumor necrosis factor alpha-mediated apoptosis and cytochrome *c* release. *Mol Cell Biol* 18: 6353–6364, 1998.
14. Brown SA, Richards CM, Hanscom HN, Feng SL, and Winkles JA. The Fn14 cytoplasmic tail binds tumour-necrosis-factor-receptor-associated factors 1, 2, 3 and 5 and mediates nuclear factor-kappaB activation. *Biochem J* 371: 395–403, 2003.
15. Cerutti PA. Prooxidant states and tumor promotion. *Science* 227: 375–381, 1985.
16. Chainy GB, Manna SK, Chaturvedi MM, and Aggarwal BB. Anethole blocks both early and late cellular responses transduced by tumor necrosis factor: effect on NF-kappaB, AP-1, JNK, MAPKK and apoptosis. *Oncogene* 19: 2943–2950, 2000.
17. Chandel NS, Schumacker PT, and Arch RH. Reactive oxygen species are downstream products of TRAF-mediated signal transduction. *J Biol Chem* 276: 42728–42736, 2001.
18. Chew LJ, Pan H, Yu J, Tian S, Huang WQ, Zhang JY, Pang S, and Li LY. A novel secreted splice variant of vascular endothelial cell growth inhibitor. *FASEB J* 16: 742–744, 2002.
19. Chicheportiche Y, Bourdon PR, Xu H, Hsu YM, Scott H, Hession C, Garcia I, and Browning JL. TWEAK, a new secreted ligand in the tumor necrosis factor family that weakly induces apoptosis. *J Biol Chem* 272: 32401–32410, 1997.
20. Chicheportiche Y, Chicheportiche R, Sizing I, Thompson J, Benjamin CB, Ambrose C, and Dayer JM. Proinflammatory activity of TWEAK on human dermal fibroblasts and synoviocytes: blocking and enhancing effects of anti-TWEAK monoclonal antibodies. *Arthritis Res* 4: 126–133, 2002.
21. Choi JH, Chung WJ, Han SJ, Lee HB, Choi IW, Lee HK, Jang KY, Lee DG, Han SS, Park KH, and Im SY. Selective involvement of reactive oxygen intermediates in platelet-activating factor-mediated activation of NF-kappaB. *Inflammation* 24: 385–398, 2000.
22. Cogswell PC, Kashatus DF, Keifer JA, Guttridge DC, Reuther JY, Bristow C, Roy S, Nicholson DW, and Baldwin AS Jr. NF-kappa B and I kappa B alpha are found in the mitochondria. Evidence for regulation of mitochondrial gene expression by NF-kappa B. *J Biol Chem* 278: 2963–2968, 2003.
23. Cohen GM. Caspases: the executioners of apoptosis. *Biochem J* 326 (Pt 1): 1–16, 1997.
24. Crawford MJ, Krishnamoorthy RR, Rudick VL, Collier RJ, Kapin M, Aggarwal BB, Al-Ubaidi MR, and Agarwal N. Bcl-2 overexpression protects photooxidative stress-induced apoptosis of photoreceptor cells via NF-kappaB preservation. *Biochem Biophys Res Commun* 281: 1304–1312, 2001.
25. Cressman DE, Greenbaum LE, DeAngelis RA, Ciliberto G, Furth EE, Poli V, and Taub R. Liver failure and defective hepatocyte regeneration in interleukin-6-deficient mice. *Science* 274: 1379–1383, 1996.
26. Czaja MJ. Induction and regulation of hepatocyte apoptosis by oxidative stress. *Antioxid Redox Signal* 4: 759–767, 2002.
27. Dypbukt JM, Ankarcona M, Burkitt M, Sjöholm A, Strom K, Orrenius S, and Nicotera P. Different prooxidant levels stimulate growth, trigger apoptosis, or produce necrosis of insulin-secreting RINm5F cells. The role of intracellular polyamines. *J Biol Chem* 269: 30553–30560, 1994.
28. Eid MA, Lewis RW, Abdel-Mageed AB, and Kumar MV. Reduced response of prostate cancer cells to TRAIL is modulated by NFkappaB-mediated inhibition of caspases and Bid activation. *Int J Oncol* 21: 111–117, 2002.
29. Feng L, Xia Y, Garcia GE, Hwang D, and Wilson CB. Involvement of reactive oxygen intermediates in cyclooxygenase-2 expression induced by interleukin-1, tumor necrosis factor-alpha, and lipopolysaccharide. *J Clin Invest* 95: 1669–1675, 1995.
30. Fernandez-Checa JC, Kaplowitz N, Garcia-Ruiz C, Colell A, Miranda M, Mari M, Ardite E, and Morales A. GSH transport in mitochondria: defense against TNF-induced oxidative stress and alcohol-induced defect. *Am J Physiol* 273: G7–G17, 1997.
31. Forsberg M, Lofgren R, Zheng L, and Stendahl O. Tumor necrosis factor-alpha potentiates CR3-induced respiratory burst by activating p38 MAP kinase in human neutrophils. *Immunology* 103: 465–472, 2001.
32. Fridovich I. The biology of oxygen radicals. *Science* 201: 875–880, 1978.

33. Galter D, Mihm S, and Droge W. Distinct effects of glutathione disulphide on the nuclear transcription factor kappa B and the activator protein-1. *Eur J Biochem* 221: 639–648, 1994.
34. Garcia-Lloret MI, Yui J, Winkler-Lowen B, and Guilbert LJ. Epidermal growth factor inhibits cytokine-induced apoptosis of primary human trophoblasts. *J Cell Physiol* 167: 324–332, 1996.
35. Garg AK and Aggarwal BB. Reactive oxygen intermediates in TNF signaling. *Mol Immunol* 39: 509–517, 2002.
36. Gergely P Jr, Niland B, Gonchoroff N, Pullmann R Jr, Phillips PE, and Perl A. Persistent mitochondrial hyperpolarization, increased reactive oxygen intermediate production, and cytoplasmic alkalinization characterize altered IL-10 signaling in patients with systemic lupus erythematosus. *J Immunol* 169: 1092–1101, 2002.
37. Ginn-Pease ME and Whisler RL. Redox signals and NF-kappaB activation in T cells. *Free Radic Biol Med* 25: 346–361, 1998.
38. Giri DK and Aggarwal BB. Constitutive activation of NF-kappaB causes resistance to apoptosis in human cutaneous T cell lymphoma HuT-78 cells. Autocrine role of tumor necrosis factor and reactive oxygen intermediates. *J Biol Chem* 273: 14008–14014, 1998.
39. Goossens V, Grooten J, De Vos K, and Fiers W. Direct evidence for tumor necrosis factor-induced mitochondrial reactive oxygen intermediates and their involvement in cytotoxicity. *Proc Natl Acad Sci U S A* 92: 8115–8119, 1995.
40. Goossens V, Grooten J, and Fiers W. The oxidative metabolism of glutamine. A modulator of reactive oxygen intermediate-mediated cytotoxicity of tumor necrosis factor in L929 fibrosarcoma cells. *J Biol Chem* 271: 192–196, 1996.
41. Goossens V, De Vos K, Vercammen D, Steemans M, Vancompernelle K, Fiers W, Vandenaabeele P, and Grooten J. Redox regulation of TNF signaling. *Biofactors* 10: 145–156, 1999.
42. Gossart S, Cambon C, Orfila C, Seguelas MH, Lepert JC, Rami J, Carre P, and Pipy B. Reactive oxygen intermediates as regulators of TNF-alpha production in rat lung inflammation induced by silica. *J Immunol* 156: 1540–1548, 1996.
43. Gottlieb E, Vander Heiden MG, and Thompson CB. Bcl-xL prevents the initial decrease in mitochondrial membrane potential and subsequent reactive oxygen species production during tumor necrosis factor alpha-induced apoptosis. *Mol Cell Biol* 20: 5680–5689, 2000.
44. Green DR and Reed JC. Mitochondria and apoptosis. *Science* 281: 1309–1312, 1998.
45. Greenlund LJ, Deckwerth TL, and Johnson EM Jr. Superoxide dismutase delays neuronal apoptosis: a role for reactive oxygen species in programmed neuronal death. *Neuron* 14: 303–315, 1995.
46. Gulbins E, Brenner B, Schlottmann K, Welsch J, Heinle H, Koppenhoefer U, Linderkamp O, Coggeshall KM, and Lang F. Fas-induced programmed cell death is mediated by a Ras-regulated O<sub>2</sub><sup>-</sup> synthesis. *Immunology* 89: 205–212, 1996.
47. Hampton MB and Orrenius S. Dual regulation of caspase activity by hydrogen peroxide: implications for apoptosis. *FEBS Lett* 414: 552–556, 1997.
48. Han S, Yoon K, Lee K, Kim K, Jang H, Lee NK, Hwang K, and Young Lee S. TNF-related weak inducer of apoptosis receptor, a TNF receptor superfamily member, activates NF-kappa B through TNF receptor-associated factors. *Biochem Biophys Res Commun* 305: 789–796, 2003.
49. Harada N, Nakayama M, Nakano H, Fukuchi Y, Yagita H, and Okumura K. Pro-inflammatory effect of TWEAK/Fn14 interaction on human umbilical vein endothelial cells. *Biochem Biophys Res Commun* 299: 488–493, 2002.
50. Haridas V, Darnay BG, Natarajan K, Heller R, and Aggarwal BB. Overexpression of the p80 TNF receptor leads to TNF-dependent apoptosis, nuclear factor-kappa B activation, and c-Jun kinase activation. *J Immunol* 160: 3152–3162, 1998.
51. Haridas V, Shrivastava A, Su J, Yu GL, Ni J, Liu D, Chen SF, Ni Y, Ruben SM, Gentz R, and Aggarwal BB. VEGI, a new member of the TNF family activates nuclear factor-kappa B and c-Jun N-terminal kinase and modulates cell growth. *Oncogene* 18: 6496–6504, 1999.
52. Hayakawa M, Miyashita H, Sakamoto I, Kitagawa M, Tanaka H, Yasuda H, Karin N, and Kikugawa K. Evidence that reactive oxygen species do not mediate NF-kappaB activation. *EMBO J* 22: 3356–3366, 2003.
53. Hietakangas V, Poukkula M, Heiskanen KM, Karvinen JT, Sistonen L, and Eriksson JE. Erythroid differentiation sensitizes K562 leukemia cells to TRAIL-induced apoptosis by downregulation of c-FLIP. *Mol Cell Biol* 23: 1278–1291, 2003.
54. Higuchi M, Aggarwal BB, and Yeh ET. Activation of CPP32-like protease in tumor necrosis factor-induced apoptosis is dependent on mitochondrial function. *J Clin Invest* 99: 1751–1758, 1997.
55. Higuchi M, Proske RJ, and Yeh ET. Inhibition of mitochondrial respiratory chain complex I by TNF results in cytochrome c release, membrane permeability transition, and apoptosis. *Oncogene* 17: 2515–2524, 1998.
56. Higuchi M, Manna SK, Sasaki R, and Aggarwal BB. Regulation of the activation of nuclear factor kappaB by mitochondrial respiratory function: evidence for the reactive oxygen species-dependent and -independent pathways. *Antioxid Redox Signal* 4: 945–955, 2002.
57. Hockenbery DM, Oltvai ZN, Yin XM, Millman CL, and Korsmeyer SJ. Bcl-2 functions in an antioxidant pathway to prevent apoptosis. *Cell* 75: 241–251, 1993.
58. Hotta T, Suzuki H, Nagai, S, Yamamoto K, Imakiire A, Takada E, Itoh M, and Mizuguchi J. Chemotherapeutic agents sensitize sarcoma cell lines to tumor necrosis factor-related apoptosis-inducing ligand-induced caspase-8 activation, apoptosis and loss of mitochondrial membrane potential. *J Orthop Res* 21: 949–957, 2003.
59. Hug H, Enari M, and Nagata S. No requirement of reactive oxygen intermediates in Fas-mediated apoptosis. *FEBS Lett* 351: 311–313, 1994.
60. Ishii Y, Partridge CA, Del Vecchio PJ, and Malik AB. Tumor necrosis factor-alpha-mediated decrease in glutathione increases the sensitivity of pulmonary vascular endothelial cells to H<sub>2</sub>O<sub>2</sub>. *J Clin Invest* 89: 794–802, 1992.
61. Itoh N and Nagata S. A novel protein domain required for apoptosis. Mutational analysis of human Fas antigen. *J Biol Chem* 268: 10932–10937, 1993.

62. Itoh N, Yonehara S, Ishii A, Yonehara M, Mizushima S, Sameshima M, Hase A, Seto Y, and Nagata S. The polypeptide encoded by the cDNA for human cell surface antigen Fas can mediate apoptosis. *Cell* 66: 233–243, 1991.
63. Jabs T. Reactive oxygen intermediates as mediators of programmed cell death in plants and animals. *Biochem Pharmacol* 57: 231–245, 1999.
64. Jones BE, Lo CR, Liu H, Pradhan Z, Garcia L, Srinivasan A, Valentino KL, and Czaja MJ. Role of caspases and NF-kappaB signaling in hydrogen peroxide- and superoxide-induced hepatocyte apoptosis. *Am J Physiol Gastrointest Liver Physiol* 278: G693–G699, 2000.
65. Kane DJ, Sarafian TA, Anton R, Hahn H, Gralla EB, Valentine JS, Ord T, and Bredesen DE. Bcl-2 inhibition of neural death: decreased generation of reactive oxygen species. *Science* 262: 1274–1277, 1993.
66. Kang HS and Choi I. Protein phosphatase 2A modulates the proliferation of human multiple myeloma cells via regulation of the production of reactive oxygen intermediates and anti-apoptotic factors. *Cell Immunol* 213: 34–44, 2001.
67. Kasof GM, Lu JJ, Liu D, Speer B, Mongan KN, Gomes BC, and Lorenzi MV. Tumor necrosis factor-alpha induces the expression of DR6, a member of the TNF receptor family, through activation of NF-kappaB. *Oncogene* 20: 7965–7975, 2001.
68. Kinscherf R, Claus R, Wagner M, Gehrke C, Kamencic H, Hou D, Nauen O, Schmiedt W, Kovacs G, Pill J, Metz J, and Daigner HP. Apoptosis caused by oxidized LDL is manganese superoxide dismutase and p53 dependent. *FASEB J* 12: 461–467, 1998.
69. Kothny-Wilkes G, Kulms D, Poppelmann B, Luger TA, Kubin M, and Schwarz T. Interleukin-1 protects transformed keratinocytes from tumor necrosis factor-related apoptosis-inducing ligand. *J Biol Chem* 273: 29247–29253, 1998.
70. Lee JR and Koretzky GA. Production of reactive oxygen intermediates following CD40 ligation correlates with c-Jun N-terminal kinase activation and IL-6 secretion in murine B lymphocytes. *Eur J Immunol* 28: 4188–4197, 1998.
71. Leroux E, Auzenne E, Weidner D, Wu ZY, Donato NJ, and Klostergaard J. Febrile and acute hyperthermia enhance TNF-induced necrosis of murine L929 fibrosarcoma cells via caspase-regulated production of reactive oxygen intermediates. *J Cell Physiol* 187: 256–263, 2001.
72. Li PF, Dietz R, and von Harsdorf R. p53 regulates mitochondrial membrane potential through reactive oxygen species and induces cytochrome *c*-independent apoptosis blocked by Bcl-2. *EMBO J* 18: 6027–6036, 1999.
73. Liu B, Andrieu-Abadie N, Levade T, Zhang P, Obeid LM, and Hannun YA. Glutathione regulation of neutral sphingomyelinase in tumor necrosis factor-alpha-induced cell death. *J Biol Chem* 273: 11313–11320, 1998.
74. Locksley RM, Killeen N, and Lenardo MJ. The TNF and TNF receptor superfamilies: integrating mammalian biology. *Cell* 104: 487–501, 2001.
75. Lotem J, Peled-Kamar M, Groner Y, and Sachs L. Cellular oxidative stress and the control of apoptosis by wild-type p53, cytotoxic compounds, and cytokines. *Proc Natl Acad Sci USA* 93: 9166–9171, 1996.
76. Manna SK, Zhang HJ, Yan T, Oberley LW, and Aggarwal BB. Overexpression of manganese superoxide dismutase suppresses tumor necrosis factor-induced apoptosis and activation of nuclear transcription factor-kappaB and activated protein-1. *J Biol Chem* 273: 13245–13254, 1998.
77. Manna SK, Kuo MT, and Aggarwal BB. Overexpression of gamma-glutamylcysteine synthetase suppresses tumor necrosis factor-induced apoptosis and activation of nuclear transcription factor-kappa B and activator protein-1. *Oncogene* 18: 4371–4382, 1999.
78. Manna SK, Haridas V, and Aggarwal BB. Bcl-x<sub>L</sub> suppresses TNF-mediated apoptosis and activation of nuclear factor-kappaB, activation protein-1, and c-Jun N-terminal kinase. *J Interferon Cytokine Res* 20: 725–735, 2000.
79. Marchetti P, Castedo M, Susin SA, Zamzami N, Hirsch T, Macho A, Haeflner A, Hirsch F, Geuskens M, and Kroemer G. Mitochondrial permeability transition is a central coordinating event of apoptosis. *J Exp Med* 184: 1155–1160, 1996.
80. Meyer M, Schreck R, and Baeuerle PA. H<sub>2</sub>O<sub>2</sub> and antioxidants have opposite effects on activation of NF-kappa B and AP-1 in intact cells: AP-1 as secondary antioxidant-responsive factor. *EMBO J* 12: 2005–2015, 1993.
81. Miao L, Yi P, Wang Y, and Wu M. Etoposide upregulates Bax-enhancing tumour necrosis factor-related apoptosis inducing ligand-mediated apoptosis in the human hepatocellular carcinoma cell line QGY-7703. *Eur J Biochem* 270: 2721–2731, 2003.
82. Morales A, Garcia-Ruiz C, Miranda M, Mari M, Colell A, Ardite E, and Fernandez-Checa JC. Tumor necrosis factor increases hepatocellular glutathione by transcriptional regulation of the heavy subunit chain of gamma-glutamylcysteine synthetase. *J Biol Chem* 272: 30371–30379, 1997.
83. Muller-Ladner U. Molecular and cellular interactions in rheumatoid synovium. *Curr Opin Rheumatol* 8: 210–220, 1996.
84. Nakamura K, Fushimi K, Kouchi H, Mihara K, Miyazaki M, Ohe T, and Namba M. Inhibitory effects of antioxidants on neonatal rat cardiac myocyte hypertrophy induced by tumor necrosis factor-alpha and angiotensin II. *Circulation* 98: 794–799, 1998.
85. Nakayama M, Ishidoh K, Kojima Y, Harada N, Kominami E, Okumura K, and Yagita H. Fibroblast growth factor-inducible 14 mediates multiple pathways of TWEAK-induced cell death. *J Immunol* 170: 341–348, 2003.
86. Newman WH, Zunzunegui RG, Warejcka DJ, Dalton ML, and Castresana MR. A reactive oxygen-generating system activates nuclear factor-kappaB and releases tumor necrosis factor-alpha in coronary smooth muscle cells. *J Surg Res* 85: 142–147, 1999.
87. Obrador E, Navarro J, Mompo J, Asensi M, Pellicer JA, and Estrela JM. Glutathione and the rate of cellular proliferation determine tumour cell sensitivity to tumour necrosis factor in vivo. *Biochem J* 325 (Pt 1): 183–189, 1997.
88. Pan G, Bauer JH, Haridas V, Wang S, Liu D, Yu G, Vincenz, C, Aggarwal BB, Ni J, and Dixit VM. Identification

- and functional characterization of DR6, a novel death domain-containing TNF receptor. *FEBS Lett* 431: 351–356, 1998.
89. Pastorino JG, Simbula G, Yamamoto K, Glascott PA Jr, Rothman RJ, and Farber JL. The cytotoxicity of tumor necrosis factor depends on induction of the mitochondrial permeability transition. *J Biol Chem* 271: 29792–29798, 1996.
  90. Perl A and Banki K. Genetic and metabolic control of the mitochondrial transmembrane potential and reactive oxygen intermediate production in HIV disease. *Antioxid Redox Signal* 2: 551–573, 2000.
  91. Phelps DT, Ferro TJ, Higgins PJ, Shankar R, Parker DM, and Johnson A. TNF- $\alpha$  induces peroxynitrite-mediated depletion of lung endothelial glutathione via protein kinase C. *Am J Physiol* 269: L551–L559, 1995.
  92. Piette J, Piret B, Bonizzi G, Schoonbroodt S, Merville MP, Legrand-Poels S, and Bours V. Multiple redox regulation in NF- $\kappa$ B transcription factor activation. *Biol Chem* 378: 1237–1245, 1997.
  93. Polek TC, Talpaz M, Darnay BG, and Spivak-Kroizman T. TWEAK mediates signal transduction and differentiation of RAW264.7 cells in the absence of Fn14/TweakR. Evidence for a second TWEAK receptor. *J Biol Chem* 278: 32317–32323, 2003.
  94. Powell CB, Scott JH, and Collins JL. Comparison of TNF- $\alpha$  and TNF $\beta$  cytolytic mechanisms in human ovarian and cervical carcinoma cell lines. *Gynecol Oncol* 71: 258–265, 1998.
  95. Pupe A, Degreef H, and Garmyn M. Induction of tumor necrosis factor- $\alpha$  by UVB: a role for reactive oxygen intermediates and eicosanoids. *Photochem Photobiol* 78: 68–74, 2003.
  96. Puskas F, Gergely P Jr, Banki K, and Perl A. Stimulation of the pentose phosphate pathway and glutathione levels by dehydroascorbate, the oxidized form of vitamin C. *FASEB J* 14: 1352–1361, 2000.
  97. Rath PC and Aggarwal BB. TNF-induced signaling in apoptosis. *J Clin Immunol* 19: 350–364, 1999.
  98. Saitoh T, Nakayama M, Nakano H, Yagita H, Yamamoto N, and Yamaoka S. TWEAK induces NF- $\kappa$ B p100 processing and long lasting NF- $\kappa$ B activation. *J Biol Chem* 278: 36005–36012, 2003.
  99. Sato K, Akaki T, and Tomioka H. Differential potentiation of anti-mycobacterial activity and reactive nitrogen intermediate-producing ability of murine peritoneal macrophages activated by interferon- $\gamma$  (IFN- $\gamma$ ) and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ). *Clin Exp Immunol* 112: 63–68, 1998.
  100. Sato N, Iwata S, Nakamura K, Hori T, Mori K, and Yodoi J. Thiol-mediated redox regulation of apoptosis. Possible roles of cellular thiols other than glutathione in T cell apoptosis. *J Immunol* 154: 3194–3203, 1995.
  101. Scarlett JL, Sheard PW, Hughes G, Ledgerwood EC, Ku HH, and Murphy MP. Changes in mitochondrial membrane potential during staurosporine-induced apoptosis in Jurkat cells. *FEBS Lett* 475: 267–272, 2000.
  102. Schenk H, Klein M, Erdbrugger W, Droge W, and Schulze-Osthoff K. Distinct effects of thioredoxin and antioxidants on the activation of transcription factors NF- $\kappa$ B and AP-1. *Proc Natl Acad Sci U S A* 91: 1672–1676, 1994.
  103. Schreck R, Rieber P, and Baeuerle PA. Reactive oxygen intermediates as apparently widely used messengers in the activation of the NF- $\kappa$ B transcription factor and HIV-1. *EMBO J* 10: 2247–2258, 1991.
  104. Schreck R, Grassmann R, Fleckenstein B, and Baeuerle PA. Antioxidants selectively suppress activation of NF- $\kappa$ B by human T-cell leukemia virus type I Tax protein. *J Virol* 66: 6288–6293, 1992.
  105. Schulze-Osthoff K, Bakker AC, Vanhaesebroeck B, Beyaert R, Jacob WA, and Fiers W. Cytotoxic activity of tumor necrosis factor is mediated by early damage of mitochondrial functions. Evidence for the involvement of mitochondrial radical generation. *J Biol Chem* 267: 5317–5323, 1992.
  106. Schulze-Osthoff K, Beyaert R, Vandevoorde V, Haegeman G, and Fiers W. Depletion of the mitochondrial electron transport abrogates the cytotoxic and gene-inductive effects of TNF. *EMBO J* 12: 3095–3104, 1993.
  107. Sen CK, Khanna S, Reznick AZ, Roy S, and Packer L. Glutathione regulation of tumor necrosis factor- $\alpha$ -induced NF- $\kappa$ B activation in skeletal muscle-derived L6 cells. *Biochem Biophys Res Commun* 237: 645–649, 1997.
  108. Shalaby MR, Aggarwal BB, Rinderknecht E, Svedersky LP, Finkle BS, and Palladino MA Jr. Activation of human polymorphonuclear neutrophil functions by interferon- $\gamma$  and tumor necrosis factors. *J Immunol* 135: 2069–2073, 1985.
  109. Shishodia S and Aggarwal BB. Nuclear factor- $\kappa$ B activation: a question of life or death. *J Biochem Mol Biol* 35: 28–40, 2002.
  110. Shoji Y, Uedono Y, Ishikura H, Takeyama N, and Tanaka T. DNA damage induced by tumor necrosis factor- $\alpha$  in L929 cells is mediated by mitochondrial oxygen radical formation. *Immunology* 84: 543–548, 1995.
  111. Shrivastava A and Aggarwal BB. Antioxidants differentially regulate activation of nuclear factor- $\kappa$ B, activator protein-1, c-Jun amino-terminal kinases, and apoptosis induced by tumor necrosis factor: evidence that JNK and NF- $\kappa$ B activation are not linked to apoptosis. *Antioxid Redox Signal* 1: 181–191, 1999.
  112. Sidoti-de Fraise C, Rincheval V, Risler Y, Mignotte B, and Vayssiere JL. TNF- $\alpha$  activates at least two apoptotic signaling cascades. *Oncogene* 17: 1639–1651, 1998.
  113. Sies H. Oxidative stress: from basic research to clinical application. *Am J Med* 91: 31S–38S, 1991.
  114. Skulachev VP. Mitochondrial physiology and pathology; concepts of programmed death of organelles, cells and organisms. *Mol Aspects Med* 20: 139–184, 1999.
  115. Soderstrom TS, Poukkula M, Holmstrom TH, Heiskanen KM, and Eriksson JE. Mitogen-activated protein kinase/extracellular signal-regulated kinase signaling in activated T cells abrogates TRAIL-induced apoptosis upstream of the mitochondrial amplification loop and caspase-8. *J Immunol* 169: 2851–2860, 2002.
  116. Staal FJ, Roederer M, and Herzenberg LA. Intracellular thiols regulate activation of nuclear factor  $\kappa$ B and transcription of human immunodeficiency virus. *Proc Natl Acad Sci U S A* 87: 9943–9947, 1990.

117. Strieter RM, Kunkel SL, and Bone RC. Role of tumor necrosis factor- $\alpha$  in disease states and inflammation. *Crit Care Med* 21: S447–S463, 1993.
118. Suliman A, Lam A, Datta R, and Srivastava RK. Intracellular mechanisms of TRAIL: apoptosis through mitochondrial-dependent and -independent pathways. *Oncogene* 20: 2122–2133, 2001.
119. Tan S, Sagara Y, Liu Y, Maher P, and Schubert D. The regulation of reactive oxygen species production during programmed cell death. *J Cell Biol* 141: 1423–1432, 1998.
120. Thurman RG. Mechanisms of hepatic toxicity. II. Alcoholic liver injury involves activation of Kupffer cells by endotoxin. *Am J Physiol* 275: G605–G611, 1998.
121. Tonks A, Cooper RA, Price AJ, Molan PC, and Jones KP. Stimulation of TNF- $\alpha$  release in monocytes by honey. *Cytokine* 14: 240–242, 2001.
122. Um HD, Orenstein JM, and Wahl SM. Fas mediates apoptosis in human monocytes by a reactive oxygen intermediate dependent pathway. *J Immunol* 156: 3469–3477, 1996.
123. Verhaegen S, McGowan AJ, Brophy AR, Fernandes RS, and Cotter TG. Inhibition of apoptosis by antioxidants in the human HL-60 leukemia cell line. *Biochem Pharmacol* 50: 1021–1029, 1995.
124. Wang JH, Redmond HP, Watson RW, and Bouchier-Hayes D. Induction of human endothelial cell apoptosis requires both heat shock and oxidative stress responses. *Am J Physiol* 272 (5 Pt 1): C1543–C1551, 1997.
125. Wiley SR and Winkles JA. TWEAK, a member of the TNF superfamily, is a multifunctional cytokine that binds the TweakR/Fn14 receptor. *Cytokine Growth Factor Rev* 14: 241–249, 2003.
126. Wong GH and Goeddel DV. Induction of manganous superoxide dismutase by tumor necrosis factor: possible protective mechanism. *Science* 242: 941–944, 1988.
127. Wyllie AH, Kerr JF, and Currie AR. Cell death: the significance of apoptosis. *Int Rev Cytol* 68: 251–306, 1980.
128. Xu Y, Bialik S, Jones BE, Iimuro Y, Kitsis RN, Srinivasan A, Brenner DA, and Czaja MJ. NF- $\kappa$ B inactivation converts a hepatocyte cell line TNF- $\alpha$  response from proliferation to apoptosis. *Am J Physiol* 275: C1058–C1066, 1998.
129. Yu J, Tian S, Metheny-Barlow L, Chew LJ, Hayes AJ, Pan H, Yu GL, and Li LY. Modulation of endothelial cell growth arrest and apoptosis by vascular endothelial growth inhibitor. *Circ Res* 89: 1161–1167, 2001.
130. Zhai D, Huang X, Han X, and Yang F. Characterization of tBid-induced cytochrome *c* release from mitochondria and liposomes. *FEBS Lett* 472: 293–296, 2000.
131. Zhang M, Wang L, Wang HW, Pan X, Pan W, and Qi ZT. Effect of N-terminal deletion on biological activity of vascular endothelial cell growth inhibitor. *Sheng Wu Hua Xue Yu Sheng Wu Wu Li Xue Bao (Shanghai)* 35: 133–137, 2003.
132. Zhang X, Chaudhry A, and Chintala SK. Inhibition of plasminogen activation protects against ganglion cell loss in a mouse model of retinal damage. *Mol Vis* 9: 238–248, 2003.

Address reprint requests to:  
 Bharat B. Aggarwal, Ph.D.  
 Cytokine Research Section  
 Department of Experimental Therapeutics  
 The University of Texas M.D. Anderson Cancer Center  
 Box 143  
 1515 Holcombe Boulevard  
 Houston, TX 77030

E-mail: aggarwal@mdanderson.org.

Received for publication September 24, 2004; accepted October 17, 2004.