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Apoptosis and Nuclear Factor-κB: A Tale of Association and Dissociation

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ABSTRACT. It is not clear why on treatment with certain killer cytokines or chemotherapeutic agents, some cells undergo apoptosis while others do not. The delineation of sensitivity/resistance pathways should provide a more specific therapy for cancer and other hyperproliferative diseases. Most cells die either by apoptosis or by necrosis. The biochemical pathway that mediates these two modes of cell death has recently been described. The nuclear factor (NF)-κB and the genes regulated by this transcription factor have been shown to play a critical role in induction of resistance to killer agents. Thus, inhibitors of NF-κB activation have a potential in overcoming resistance to apoptosis induced by various agents. The evidence for and against such a notion is discussed. BIOCHEM PHARMACOL **60**;8:1033–1039, 2000. © 2000 Elsevier Science Inc.

KEY WORDS. NF-κB; apoptosis; caspases; reactive oxygen intermediates; TNF

Cancer is a hyperproliferative disorder resulting from the inability of cells to undergo normal cell death. Typically, these cells die either by necrosis or apoptosis (also called programmed cell death). Necrosis is characterized by loss of plasma membrane integrity without severe damage to the nucleus, whereas apoptosis occurs by organized degradation of subcellular components. Apoptosis is distinguished by structural and morphological features involving cell shrinkage, plasma membrane blebbing, mitochondrial swelling, and release of cytochrome c, chromatin condensation, and DNA fragmentation [1].

Among all the known physiological inducers of apoptosis in mammalian cells, TNF† is perhaps the most potent and well studied. Many other members of the TNF superfamily also induce apoptosis, including LT (lymphotoxin), FasL (fibroblast-associated ligand), TRAIL (TNF-related apoptosis-inducing ligand), DR3L (for death receptor 3 ligand or also known as TWEAK for a weak homologue of TNF), THANK (TNF homologue that activates apoptosis, NF-κB, and c-Jun N-terminal kinase), and VEGI (vascular endothelial cell growth inhibitor) [2, 3, and references therein]. Whether all these TNF family members induce apoptosis by the same mechanism as TNF is not known. Besides killer cytokines outlined above, apoptosis is also induced by various chemotherapeutic agents.

Within the last few years, a series of biochemical steps has been identified that results in apoptosis by cytokines and chemotherapeutic agents (Fig. 1). For instance, TNF-induced apoptosis involves activation of the TNF receptor, which, through its cytoplasmic death domain, recruits a protein called TNF receptor-associated death domain TRADD, which in turn sequentially recruits Fas-associated death domain (FADD) and FADD-like ICE (FLICE, also called caspase-8) [for references see 4–6]. The latter activates caspase-9, which in turn activates caspase-3 (the executioner protease), resulting in apoptosis. In contrast to cytokines, chemotherapeutic agents induce cellular apoptosis by inducing formation of mitochondrial transition pores, a rapid decrease in the mitochondrial transmembrane potential, and release of cytochrome c. The latter, in the presence of the protein apoptosis protease-activating factor 1 (Apaf-1), activates caspase-9, which then activates caspase-3. Several recent studies, however, have suggested that these two receptor-mediated and non-receptor-mediated pathways initiated by cytokines and chemotherapeutic agents, respectively, are not exclusive of each other and share similar steps (Fig. 1).

Most agents that induce apoptosis also activate NF-κB (Fig. 1). Thus, it is not too surprising that almost all cytokines of the TNF superfamily and chemotherapeutic agents activate NF-κB. TNF-induced activation of NF-κB (primarily consisting of p50 and p65 subunits) involves recruitment of TRAF2 by TRADD, which then binds to NIK. TRADD also binds to RIP. Either NIK or RIP then activate a kinase called IκBα kinases (IKK), which in turn leads to the phosphorylation, ubiqutination, and degradation of IκBα (the inhibitory subunit of NF-κB), leading to NF-κB activation [6]. There are some recent studies which exclude the role of NIK in TNF-induced NF-κB activation [for references see 6]. The manner in which chemothera-

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† *Abbreviations:* TNF, tumor necrosis factor; NF-κB, nuclear factor kappa B; TRADD, TNF receptor associated death domain; TRAF2, TNF receptor associated factor 2; SOD, superoxide dismutase; RIP, receptor-interacting protein; and IκBα, inhibitor kappaBalpha.

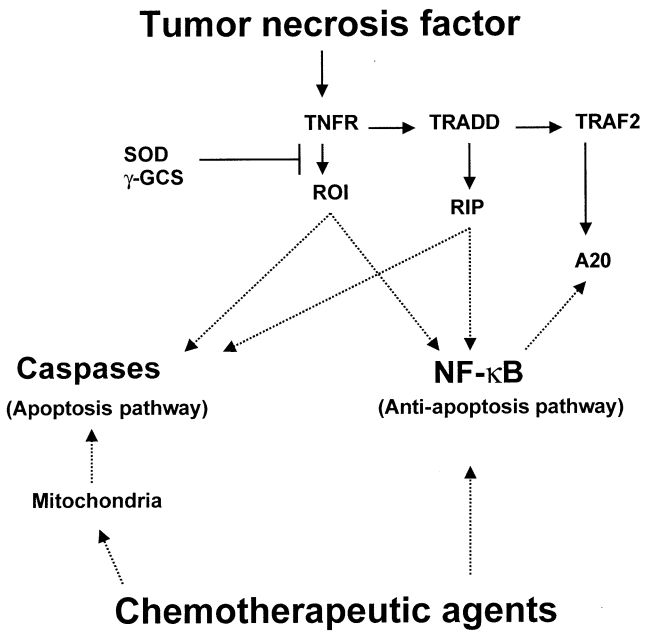

FIG. 1. Mechanism of activation of NF-κB and apoptosis by cytokines and chemotherapeutic agents. TNFR, TNF receptor; ROI, reactive oxygen intermediate; and SOD, superoxide dismutase.

peutic agents activate NF-κB is not fully understood, but most likely also involves phosphorylation, ubiqitination, and degradation of IκBα [6]. How NF-κB activation is linked to induction of apoptosis by TNF and chemotherapeutic agents is the subject of this review. That apoptosis is blocked by NF-κB activation has been reviewed [7, 8].

NEGATIVE ASSOCIATION OF NF-κB WITH APOPTOSIS

It has been known for several years that the cytotoxic effects of TNF are enhanced by the presence of inhibitors of protein synthesis [9 and references therein]. The mechanism of this enhancement, however, is not understood. It is also known that pretreatment of cells with lymphotoxin induces, by some unknown mechanism, cellular resistance to the subsequent treatment with the same cytokine [10]. In 1996, five independent reports appeared, all of which indicated that activation of NF-κB induces resistance to apoptosis [11–15]. These concluded that treatment of RelA-deficient (the transcriptionally active subunit of NF-κB) mouse fibroblasts and macrophages with TNF significantly reduced the cell viability, whereas RelA+/+ cells were unaffected. In addition, reintroduction of RelA into RelA−/− fibroblasts enhanced survival, demonstrating that Rel A is required for protection from TNF [11]. Another report showed that the activation of NF-κB by TNF, ionizing radiation, or daunorubicin protects cells from apoptosis, whereas inhibition of NF-κB enhanced apoptotic killing by these reagents but not by apoptotic stimuli that do not activate NF-κB [12]. Van Antwerp *et al.* [13] took a different approach and showed that the sensitivity and kinetics of TNF-induced apoptosis are enhanced in a number of cell types expressing a dominant negative IκBα (an inhibitor of NF-κB). Liu *et al.* [14] used the signaling proteins and showed that recruitment of Fas-associated death domain (FADD) to the TNFR1 complex mediates apoptosis and the recruitment of RIP and TRAF2 mediates NF-κB activation, and that the activation of the latter protects cells against TNF-induced apoptosis. Another study showed that the treatment of WEH1 231 cells with *N*-tosyl-L-phenylalanine chloromethyl ketone (TPCK), a protease inhibitor that prevents degradation of IκBα, or with low doses of pyrrolidine dithiocarbamate (PDTC) selectively inhibited NF-κB activation and induced apoptosis [15]. Similarly, microinjection of WEHI 231 cells with either IκBα–glutathione *S*-transferase protein or a c-Rel affinity-purified antibody induced apoptosis [15]. All these reports strongly suggest that activation of NF-κB blocks TNF-induced apoptosis and suppression of NF-κB activation potentiates TNF-induced apoptosis.

That NF-κB activation can block TNF-induced apoptosis is not restricted to a few cell types, but has been noted in a variety of cell types including fibrosarcoma HT 1080 [16], keratinocytes [17], endothelial cells [18, 19], myeloid cells [20], chronic lymphocytic leukemia [21], lymphoid cell lines [22], cutaneous T cell lymphoma [23], hepatocyte cell lines [24], melanoma cells [25], prostate cancer cells [26], epithelial cells [27], glomerular mesangial cells [28], pancreatic cancer cells [29], Ewing tumor cells [30], and head and neck squamous cell carcinoma [31]. In almost all these cell types, suppression of NF-κB either by metabolic inhibitors, antioxidants, or the dominant negative form of IκBα reverses the sensitivity of cells to TNF.

Additional support for the role of NF-κB in apoptosis stems from the studies in which agents that block NF-κB activation potentiated TNF-induced apoptosis and those that activate NF-κB blocked the apoptosis. For instance Par-4, a prostate cancer cell-specific gene product, potentiates TNF-induced apoptosis by suppressing NF-κB activation through inactivation of atypical protein kinase C [32]. *Yersinia enterocolitica* impaired the activation of NF-κB, which led to enhanced TNF-induced apoptosis in HeLa cells [33]. Lipopolysaccharides blocked TNF-induced apoptosis in myeloid cells through activation of NF-κB [34], as did hepatitis C core protein [35, 36]. In agreement with the negative role of NF-κB in apoptosis, protein kinase C-mu down-regulated TNF-induced apoptosis, and this correlated with enhanced expression of NF-κB-dependent protective genes [37]. It is known that adenovirus protein E1B blocks TNF-induced apoptosis, whereas E1A enhances TNF-induced apoptosis through unknown mechanisms. Recent evidence indicates that the effect of these proteins is mediated through modulation of NF-κB activation [38].

POSSIBLE MECHANISMS BY WHICH NF-κB NEGATIVELY REGULATES APOPTOSIS

Although it is clear that NF-κB activation may play a role in suppressing TNF-induced apoptosis, just how is still not fully understood. Several genes that may play a role in blocking apoptosis and whose expression is regulated by NF-κB have been identified. These include cellular inhibitors of apoptosis (cIAP)-1, cIAP-2, TRAF1, and TRAF2 [19, 39, 40]. The cIAP-1, cIAP-2, and TRAF1 are known to bind to TRAF2, and TRAF2 is required for NF-κB activation. Thus, how these proteins play a role in blocking apoptosis is not clear. Other reports show that TNF induces manganous superoxide dismutase (SOD), whose expression is also regulated by NF-κB, and that the overexpression of SOD induces resistance to TNF-induced apoptosis [41]. Insulin manifests its antiapoptotic signaling though the activation of the NF-κB-dependent survival genes encoding TRAF2 and SOD [42]. The TNF-inducible zinc finger protein A20 [43] is regulated by NF-κB [44], and the role of this protein in induction of resistance to TNF-induced apoptosis has been demonstrated [45]. The expression of a protein critical for the regulation of the cell cycle, cyclin D1, is also regulated by NF-κB, and this may contribute to the cell growth and differentiation activity assigned to NF-κB [46, 47].

The prosurvival Bcl-2 homologue Bfl-1/A1 is another gene whose transcription is regulated by NF-κB and which blocks TNF-induced apoptosis [16, 48]. There are other studies which show that Bcl-2 activates NF-κB through the degradation of the inhibitor IκBα [49]. It is known that the Ras/phosphatidylinositol-3 kinase/Akt pathway plays a critical role in cell survival. It now appears that this pathway is linked to the activation of IKK, the kinase needed for IκBα phosphorylation and NF-κB activation. Akt may play a cytoprotective role, also through activation of NF-κB [50]. An NF-κB-independent cytoprotective pathway has also been described. The NF-κB activation induced by overexpression of TRAF2 was found to be insufficient to protect cells from apoptosis induced by TNF and cycloheximide together, thus indicating an essential role for additional components in the cytoprotective response [51].

While NF-κB activation blocks apoptosis, it seems that activation of apoptosis also blocks NF-κB activation, suggesting a feedback loop. For instance, endothelial cells undergo apoptosis when deprived of growth factors. The surviving viable cells exhibit increased activity of NF-κB, whereas apoptotic cells show caspase-mediated cleavage of the NF-κB p65/ReIA subunit, resulting in loss of carboxy-terminal transactivation domains and a transcriptionally inactive p65 molecule, which itself acts as a dominant negative inhibitor of NF-κB, promoting apoptosis. In contrast, an uncleavable, caspase-resistant p65 protects the cells from apoptosis. The generation of a dominant negative fragment of p65 during apoptosis may be an efficient proapoptotic feedback mechanism between caspase activation and NF-κB inactivation [52]. Similarly, apoptosis has been shown to promote a caspase-induced amino-terminal truncation of IκBα that functions as a stable inhibitor of NF-κB [53], thus further enhancing apoptosis. Similarly, Fas, another member of the TNF receptor family, was found to induce caspase-3-mediated proteolysis of both p50 and p65 subunits of NF-κB in Jurkat T cells, thus sensitizing the cells to apoptosis [54].

POSITIVE ASSOCIATION OF NF-κB WITH APOPTOSIS

While there are several reports that NF-κB activation protects cells from undergoing apoptosis induced by TNF or chemotherapeutic agents, there are also reports suggesting that NF-κB activation mediates apoptosis. For instance, in murine clonal osteoblasts NF-κB activation mediated TNF-induced apoptosis [55]. The suppression of growth of CD34+ myeloid cells by TNF also correlated with NF-κB activation [56]. Similarly, H_2O_2-induced apoptosis was not suppressed by hyperoxia-induced NF-κB activation [57]. In pancreatic islets, A20 inhibited both apoptosis and NF-κB activation induced by cytokines, suggesting that NF-κB may actually mediate apoptosis [58]. Apoptosis in HL-60 cells induced by chemotherapeutic agents such as etoposide or 1-beta-D-arabinofuranosylcytosine was also found to require NF-κB activation, inasmuch as suppression of NF-κB by pyrrolidine dithiocarbamate also blocked apoptosis [59]. That the activation of NF-κB is instead required for apoptosis has also been shown for other inducers such as H_2O_2 [60, 61]. The apoptosis induced by alphavirus was also found to require the activation of NF-κB [62], since the thiol agents and Bcl-2 blocked both the activities. Apoptosis in Ca^{2+} reperfusion injury of cultured astrocytes was also found to be mediated through NF-κB activation [63]. The cell death-promoting role of NF-κB has also been demonstrated in focal cerebral malaria [64]. Lin *et al.* [65] showed that NF-κB can be pro- or antiapoptotic depending on the timing of modulating NF-κB activity relative to the death stimulus. Thus, these observations suggest that NF-κB activation not only negatively but also positively regulates apoptosis. How NF-κB may mediate apoptosis is not clear, but the role of p53 and c-Myc induction through NF-κB has been demonstrated [66]. The role of c-Myc has also been implicated in survival of certain cells such as hepatocytes [67].

DISSOCIATION OF NF-κB FROM APOPTOSIS

Several reports have indicated that NF-κB activation has no effect on apoptosis induced by various cytokines and chemotherapeutic agents. Among all the cytokines that induce apoptosis, TNF is the only one whose apoptosis is affected by NF-κB. FasL (Fas-associated ligand) and TRAIL (TNF-related apoptosis-inducing ligand) the other two members of the TNF family, are potent inducers of apoptosis, but apoptosis induced by them is not affected by NF-κB activation. Even in the case of TNF, Cai et al. [68] showed

that IκBα overexpression in human breast carcinoma MCF7 cells inhibits NF-κB activation but not TNF-induced apoptosis. Similarly, in endothelial cells A20 inhibited NF-κB activation without enhancing TNF-induced apoptosis [69]. Lipopolysaccharides and interleukin-1 induced survival of endothelial cells but did not require NF-κB activation [18]. Similarly, the apoptosis induced by only a very limited number of chemotherapeutic agents is down-regulated by NF-κB. For instance, Wang *et al.* reported that apoptosis induced by daunorubicin is inhibited by NF-κB [12]. The same group later reported that camptothecin (CPT11)-induced apoptosis is also blocked by NF-κB [16]. Other reports have indicated that NF-κB activation blocks apoptosis induced by taxol, but had no effect on apoptosis induced by daunomycin, vincristine, and vinblastin [23, 35]. Instead, etoposide- or ara C-mediated apoptosis was actually found to require NF-κB activation [59, 70, 71]. Why NF-κB plays a role in apoptosis induced by some agents and not others is not clear, but suggests that the apoptotic pathway varies from one inducer to another.

LINK BETWEEN NF-κB AND APOPTOSIS IS PARADOXICAL

Various lines of evidence suggest that the relationship between NF-κB and apoptosis is quite complex. Most agents, including cytokines and chemotherapeutic agents, activate both NF-κB and apoptosis (Fig. 1). Invariably, NF-κB activation precedes apoptosis. This suggests that NF-κB may mediate rather than block subsequent apoptosis. Why NF-κB activated by an agent such as TNF is not sufficient to block the apoptosis induced by the same agent is not clear. Second, not only extracellular stimuli but also intracellular signaling proteins mediate both TNF-induced apoptosis and NF-κB activation. For instance, RIP1 and RIP2 mediate both TNF-induced apoptosis and NF-κB activation through their death domains [72–74]. RIP3, which lacks a death domain, can also activate both NF-κB and apoptosis [75]. Nod1 is a leucine-rich repeat-containing apoptosis protease-activating factor 1 (Apaf-1)-like molecule that can regulate both apoptosis and NF-κB activation pathways [76]. CLAP, another novel caspase recruitment domain-containing protein in the TNF receptor pathway, regulates NF-κB activation and apoptosis [77]. The deletion of IKKβ, however, a kinase needed for TNF-induced NF-κB activation, abolishes NF-κB activation but enhances apoptosis [78]. Third, evidence from our laboratory and others has suggested that reactive oxygen intermediates (ROI) are required for activation of both NF-κB and apoptosis (Fig. 1). Thus, overexpression of either manganese superoxide dismutase or γ-glutamyl cysteine synthetase suppresses TNF-induced NF-κB activation and apoptosis [41, 79]. Similarly, Bcl-2 and Bcl-xL, which block apoptosis, have also been shown to suppress NF-κB activation induced by TNF in endothelial cells [80].

Considering all these factors, it appears that the role of NF-κB in apoptosis is quite complex and is determined by the experimental conditions, including the cell type, the inducer of apoptosis, the inhibitor of NF-κB, and the kinetics. For instance, TNF induces NF-κB activation in almost all cell types within minutes, but not all cell types undergo apoptosis in response to TNF. In fact, very few cell types are killed by TNF and it usually takes 24–72 hr before apoptosis occurs. Thus, it seems that NF-κB activation alone is not sufficient to induce cell survival or apoptosis. If we assume that the cell is well-equipped with survival instincts and that there are proteins that contribute to its survival process, these proteins are most likely regulated not only by NF-κB but also by various other transcription factors. Similarly, another set of proteins mediates cell death. Whether the cell undergoes cell death or proliferation or is unaffected in response to a given agent may depend on the pre-existing balance between survival and antisurvival proteins. This postulate might explain why NF-κB displays opposite activities with respect to the regulation of apoptosis. The reports indicated above also suggest that both NF-κB and apoptosis share an overlapping upstream signaling pathway and that this pathway may differ downstream.

SIGNIFICANCE AND CONCLUSION

The overall evidence presented above suggests that NF-κB could be associated with apoptosis in some situations, either in a negative or positive fashion, and dissociated from apoptosis in other situations. The possibility that NF-κB activation plays a role in hyperproliferative disorders such as cancer has potential in designing better therapy. The negative role of NF-κB activation was demonstrated not only *in vitro* but also *in vivo*. Cancer patients quite frequently develop resistance to various chemotherapeutic agents, with some recent evidence suggesting the role of NF-κB. For instance, Wang *et al.* [16] showed that inhibition of NF-κB activation enhanced chemotherapy-induced tumor regression. NF-κB suppression also enhanced the sensitivity of UV-induced apoptosis in squamous cell carcinoma [81]. Similarly, in acute myeloid leukemia or in T cell lymphoma where interleukin-1 and TNF, respectively, act as an autocrine growth factor through constitutively active NF-κB, suppression of NF-κB has inhibited the growth of these tumors [23, 82]. Thus, NF-κB could be used as a reasonable target to enhance chemotherapy-induced tumor regression in cancer patients in some selective situations.

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