Apoptosis: **Activate NF-κB or die?**

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Activation of the transcription factor NF-κB has been linked to apoptosis, with the factor playing either an anti-apoptotic or a pro-apoptotic role, depending on the type of cell in which it is expressed.

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The transcription factor NF-κB has for some years been recognized to be a central mediator of the gene expression induced in cells by pathogens or inflammatory cytokines, and is known to play an important role in development of the immune system [1]. To the list of NF-κB's biological activities can now be added a role in countering the induction of apoptosis (or programmed cell death) by the cytokine tumor necrosis factor-α (TNF-α), ionizing radiation or the cancer chemotherapeutic agent daunorubicin [2–6].

The form of active cell death known as apoptosis is an important process in embryogenesis and lymphoid cell development that results in selective elimination of cells from the body. Once the decision to undergo cellular suicide has been made, a proteolytic cascade is triggered in the cell, which ultimately results in activation of nucleases that degrade chromosomal DNA [7]. Cells exposed to DNA-damaging agents or TNF-α, treatments that are known to induce NF-κB activation, in combination with a protein or RNA synthesis inhibitor also undergo apoptosis. Several recent papers [2–6] have now shown that activation of NF-κB by these agents is part of a regulatory loop that operates to prevent cell death, most likely by inducing the expression of anti-apoptotic genes.

An inkling of a connection between NF-κB and cell death came from studies on 'knockout' mice that lack the 65 kDa RelA subunit (also known as p65) of NF-κB as a result of targeted mutation of the *relA* gene. These mice die before birth and show massive degeneration of liver cells caused by apoptosis, suggesting that, in mice, NF-κB has a protective role, at least during early liver development [8]. Beg and Baltimore [2] have now demonstrated that fibroblasts and macrophages from the RelA-deficient mice are sensitized to TNF-α-induced cytotoxicity and die within eight hours of exposure to the cytokine, whereas wild-type cells survive the treatment. The susceptibility of the RelA-deficient cells to TNF-α-induced toxicity is reversed following transfection of the cells with the wild-type *relA* gene, arguing against the view that the cells suffer a gross developmental defect that predisposes them to apoptosis.

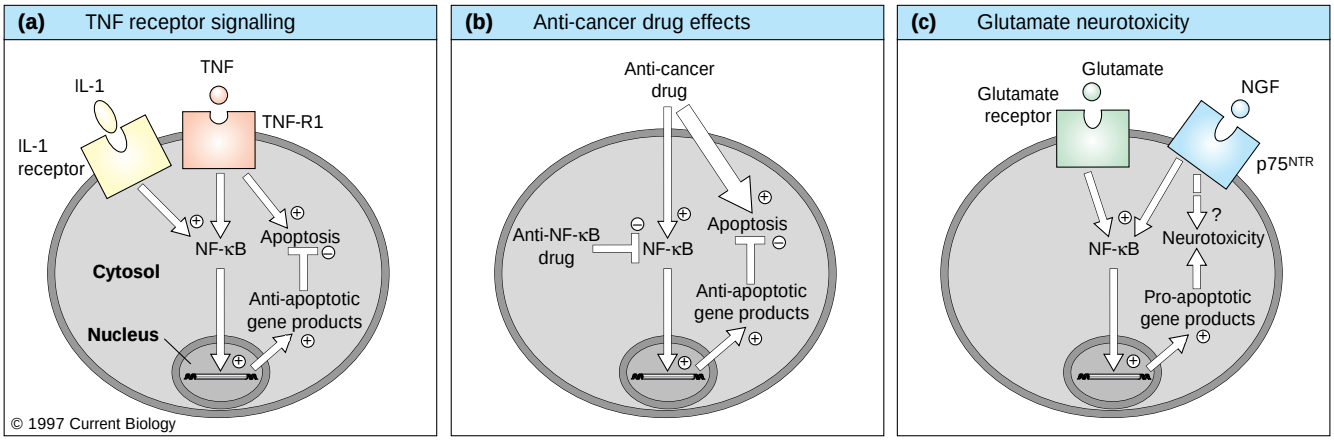
It had previously been shown that cells from the *relA* knockout mice do not respond to TNF-α by activation of NF-κB or the induction of normally TNF-α-responsive genes with NF-κB-binding (κB) promoter sites [2]. The simplest conclusion from these observations is that one or more of the NF-κB-dependent genes that are induced by TNF-α protect cells from apoptosis. Such a scenario also helps explain why, in most cell types, TNF-α is not cytotoxic unless the cells are simultaneously exposed to an inhibitor of RNA or protein synthesis, which may block expression of NF-κB-dependent genes that have a protective function (see Fig. 1a).

A requirement of NF-κB for preventing apoptosis has also been shown by functionally inactivating the NF-κB transcription factor in RelA-expressing cells [3–7]. Prototypical NF-κB, a heterodimer of RelA and 50 kDa subunit (p50), is usually retained in the cytoplasm of unstimulated cells in an inactive form by members of the inhibitory protein (IκB) family. Following cell stimulation, IκB-α is rapidly phosphorylated at two serine residues near its amino terminus, which targets this subunit for proteolytic degradation. The released NF-κB can then translocate to the nucleus, where it activates the transcription of target genes. Mutation of IκB-α so that it can no longer be inducibly phosphorylated creates a dominant-negative form that, when expressed in cells, potently suppresses activation of the entire NF-κB family.

Several groups have demonstrated that cells expressing such a dominant-negative mutant form of IκB-α are killed by TNF-α, even in the absence of a protein synthesis inhibitor [3–6]. Conversely, pretreatment of cells with interleukin 1β (IL-1β), a cytokine that activates NF-κB but is not pro-apoptotic, protects them from apoptosis induced by the later addition of TNF-α plus a protein synthesis inhibitor. In cells already expressing the dominant-negative IκB-α, IL-1β does not have a protective effect, suggesting that the effect of IL-1β also relies on the induction of NF-κB-controlled anti-apoptotic genes.

A role for NF-κB in preventing apoptosis is also evident in B-lymphocyte cell lines. Such cell lines express constitutively active NF-κB, and inactivation of the factor by various means induces apoptosis [6]. When murine B lymphoma cells are treated with protease inhibitors or antioxidant compounds known to inhibit NF-κB activation,

Figure 1



Roles of NF-κB in the regulation of cell death. The cell type and stimulus determines whether NF-κB activation protects cells from apoptosis or promotes cell death. (**a**) Binding of TNF-α to TNF receptor subtype 1 (TNF-R1) can trigger both NF-κB activation and apoptosis. Activated NF-κB induces the expression of gene products that block the apoptotic pathway. Preventing the induction of the anti-apoptotic genes by interfering with protein or RNA synthesis, or by inactivating NF-κB functionally, genetically or pharmacologically, promotes apoptosis. IL-1 can only induce NF-κB, and so pretreating cells with IL-1 makes them more resistant to apoptotic signals. (**b**) Several anti-cancer drugs activate NF-κB in addition to inducing cell death. NF-κB activation may counteract the therapeutic effects of these compounds, and combining anti-cancer therapies with NF-κB blocking agents might result in more effective anti-cancer treatments. (**c**) In neuronal cells, glutamate-induced toxicity is accompanied by NF-κB activation. However, NF-κB may induce the expression of pro-apoptotic genes in these cells to facilitate cell death. Binding of NGF to its low-affinity receptor $p75^{NTR}$ on Schwann cells results in NF-κB activation. The relationship, if any, between NF-κB activation and cell death triggered by $p75^{NTR}$ activation in these cells is not established.

or microinjected with IκB-α, they die showing features characteristic of apoptosis. Thus, in B cells, constitutive NF-κB has an role in ensuring cell survival, and thereby cell proliferation. As B-cell proliferation is exquisitely stimulated by pro-inflammatory conditions, constitutive NF-κB activation in this cell type may have evolved to ensure a more permanent protection of cells from the enhanced levels of pro-apoptotic cytokines present in inflamed areas of the organism.

Wang *et al.* [3] have demonstrated that cells with inactivated NF-κB are also more prone to commit suicide in response to pro-apoptotic stimuli other than the inflammatory cytokine TNF-α. DNA-damaging agents, such as ionizing radiation and daunorubicin, which can also activate NF-κB, are more toxic to cells when NF-κB activation is blocked by expression of a dominant-negative IκB-α mutant. An important therapeutic implication of these observations is that it may be possible to enhance the efficacy of anti-cancer drugs by inhibiting NF-κB activation. Several anti-cancer compounds are cytotoxic and kill tumor cells by inducing apoptosis. By combining cancer chemotherapeutics with a compound that blocks NF-κB activation their anti-tumor activity may be enhanced (see Fig. 1b).

The putative anti-apoptotic genes that are activated by NF-κB in response to endogenous TNF-α and exogenous genotoxic and stress-inducing conditions remain to be identified. Two likely candidates are the genes for manganese superoxide dismutase and the zinc finger protein A20. Expression of both genes is induced by TNF-α and the two protein products provide partial protection against apoptosis when expressed constitutively [9,10]. In the case of A20, NF-κB was identified as a key transcriptional regulator of its expression. However, a role of NF-κB in regulation of manganese superoxide dismutase gene expression has not been established yet.

Multicellular organisms appear to have evolved an intricate strategy of linking NF-κB activation with protection from apoptosis. A major purpose of this connection may be to avoid unnecessary cell suicide in response to certain pro-apoptotic signals, such as TNF-α. Only when TNF-α hits a cell that is incapable of mounting a NF-κB-dependent genetic response — because the cell is damaged or its protein or RNA synthesis is inhibited by small molecules or a viral mechanism — would the cell be killed by an otherwise tolerable stimulus. This way, the organism can screen itself for the presence of damaged or infected cells and eliminate them while the transcriptionally and translationally functional cells would stay alive. NF-κB is thus a key regulator that connects the execution of the apoptotic program with an interpretation of the functional state of the cell's protein expression machinery.

NF-κB plays a pivotal role in defending the organism against viruses by inducing the expression of various antiviral proteins in response to viral proteins or viral RNA intermediates. One way for viruses to subvert this control would be to block NF-κB activation by some

NF-κB-inhibitory mechanism acquired during their evolution, as is the case with the African Swine Fever Virus, the genome of which actually encodes an IκB-like inhibitor. In such a situation, however, the virus-infected cell with an inactivated NF-κB would become more sensitive to stimuli that induce apoptotic death, such as TNF-α, particularly once the virus attempts to subvert and control the cell's protein expression machinery.

A general role for NF-κB as a transcription factor that prevents cell death is, however, far from established. A recent study with neurons suggests that NF-κB can have the opposite role [11]. Glutamate-induced toxicity in neuronal cells was found to be accompanied by the induction of NF-κB. In this case, NF-κB activation appears to cause cell death, as blocking its activation with aspirin or salicylate protected the cells from the neurotoxic effects of glutamate [11]. If this study can be extended to other NF-κB-activating neurotoxins, such as β amyloid peptide, then it seems that, in neurons, unlike several other cell types, NF-κB maybe involved in triggering cell death (Fig. 1c). In Schwann cells, nerve growth factor (NGF) has been shown to activate NF-κB through the neurotrophin receptor p75 [12]. It would be interesting to determine if NF-κB performs a pro-apoptotic or anti-apoptotic role in this glial cell type, as the p75 receptor is believed to mediate both cell survival and cell death, depending on the developmental state of neurons and the amount of receptor expressed.

Two other reports have also implicated NF-κB in promoting apoptosis [13,14]. In one study [13], apoptotic death induced in a human embryonic kidney cell line (293 cells) by serum withdrawal was shown to be accompanied by NF-κB activation. Interestingly, transient or stable overexpression of *bcl-2*, the product of which is known to interfere with apoptosis, reduced κB-dependent reporter gene expression and prevented cell death. Conversely, overexpression of a dominant-negative form of RelA, capable of suppressing κB-dependent gene expression, resulted in partial inhibition of apoptosis. In the other study [14], Sindbis-Virus-induced apoptosis in a carcinoma cell line was similarly shown to require NF-κB activation. Thus, there are clearly situations in which NF-κB activation appears to be required for apoptosis to occur. In this respect, it is interesting to note that the list of target genes for NF-κB include both anti-apoptotic (see above) and pro-apoptotic genes, such as c-*myc* and *p53* [1].

The role of NF-κB as a promoter or attenuator of cell death may ultimately depend on both the cell type and the nature of the apoptosis-inducing stimulus. In different cell types, NF-κB could perform these opposing functions by activating distinct patterns of genes in conjunction with cell-type-specific transcription factors. Apoptosis-inducing stimuli may differ with respect to the kind of pathways they can activate in addition to the ones controlling apoptosis and NF-κB. Additional activated transcription factors, such as AP-1 or Ets factors, may further influence the spectrum of induced genes to determine whether NF-κB can oppose or promote apoptosis. NF-κB appears to be used as a genetic switch whose biological effect is determined by the set of genes it can access in a given cell type.

An interesting consequence of the recently uncovered anti-apoptotic role of NF-κB is that it may be difficult pharmacologically to block NF-κB-activating pathways without increasing a cell's susceptibility to cytotoxic stimuli. While this may be desirable when treating tumors with cytotoxic chemotherapeutics, it may not be advantageous when treating chronic inflammatory diseases with NF-κB inhibitors. A number of drugs that treat rheumatoid arthritis were recently recognized to be inhibitors of NF-κB, including glucocorticoids, salicylates and gold compounds [15]. A characteristic of glucocorticoids and salicylates is that they only achieve a partial inhibition of NF-κB which, while still allowing for a protection from apoptosis, may be sufficient to significantly reduce the production of inflammatory cell-adhesion molecules and cytokines. The future development of anti-inflammatory drugs that target NF-κB, and improved chemotherapeutic treatments, will certainly benefit from the recent findings that prompted this commentary.

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