NF-κB at the crossroads of life and death

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The choice between life and death is one of the major events in regulation of the immune system. T cells that specifically recognize viral or bacterial antigens are selected to survive and proliferate in response to infection, whereas those that are self-reactive are eliminated *via* apoptosis. Even the survival of alloreactive T cells requires their proper costimulation and, when infection subsides, the activated T cells are eliminated. A major regulator of such life or death decisions is the transcription factor NF-κB. However, NF-κB cannot function alone. A variety of mechanisms exist to modulate its activity and thereby affect the ultimate outcome of a cell's fate.

The DNA-binding activity of NF-κB is rapidly induced in all cell types in response to proinflammatory cytokines and the byproducts of microbial and viral infections. It was therefore anticipated that NF-κB–induced transcription would play a central role in host defense and inflammatory responses. These predictions were bolstered by the identification of NF-κB–binding sites in the promoter regions of genes encoding a plethora of cytokines, chemokines, adhesion molecules and enzymes that produce secondary inflammatory mediators[1,2]. The generation of mouse models that show defective NF-κB activation or lack essential NF-κB subunits, such as RelA (also known as p65), has proven the importance of NF-κB in innate and adaptive immunity, inflammation and lymphoid organ development[3–7]. The analysis of NF-κB–deficient mice and cells also led to the identification of another function of this versatile transcription factor: the inhibition of apoptosis[8–11]. As we shall argue here, the ability of NF-κB to prevent the induction of programmed cell death by a variety of proinflammatory and innate immune stimuli is tightly linked to its essential proinflammatory activity.

Rather than dwell on the aspects of NF-κB regulation and function that have been reviewed already[12], we will focus here on insights into the anti-apoptotic function of NF-κB and its regulation. NF-κB is a collective term referring to dimeric transcription factors that belong to the Rel family and are regulated *via* shuttling from the cytoplasm to the nucleus in response to cell stimulation[13]. Mammals express five Rel (NF-κB) proteins that belong to two classes. The first class includes RelA, c-Rel and RelB, proteins that are synthesized as mature products and do not require proteolytic processing. The second group is encoded by the *Nfkb1* and *Nfkb2* genes, whose products are first synthesized as large precursors, p105 and p100, respectively, that require proteolytic processing to produce the mature p50 and p52 NF-κB proteins. NF-κB dimers containing RelA or c-Rel, are held in the cytoplasm through interaction with specific inhibitors, the IκBs. The IκBs undergo rapid ubiquitin-dependent degradation after exposure to a variety of agonists, which activate the IκB kinase (IKK) complex[12].

IKK is composed of two catalytic subunits, IKKα and IKKβ, which can directly phosphorylate IκB, and a regulatory subunit, IKKγ (also known as NEMO), whose integrity is required for the activation of this pathway (**Fig. 1**). At least one NF-κB dimer, formed between RelB and p52, is subject to a different form of regulation. In the cytoplasm of nonstimulated cells, RelB is associated with the NF-κB2 p100 polypeptide whose IκB-like COOH-terminus is degraded after cell stimulation to release RelB-p52 dimers that translocate to the nucleus[14]. Activation of this process is dependent on one of the IKK subunits, IKKα[15], whereas activation of the canonical NF-κB pathway is mostly dependent on IKKβ[16–18]. Whereas the canonical NF-κB pathway, which is based on IκB degradation, is essential for innate immunity[7], the second pathway, which is based on NF-κB2 processing, is mostly involved in lymphoid organ development and adaptive immunity[15]. Once in the nucleus, NF-κB dimers are subject to further regulation mainly through phosphorylation of the Rel proteins, which is required for full induction of NF-κB target genes[19,20]. Several signaling pathways, which induce the phosphatidyl inositol 3 kinase (PI3K)→Akt pathway, are thought to be involved in this process[21].

NF-κB as an anti-apoptotic transcription factor

The first indication that suppression of apoptosis is an important NF-κB function came from the analysis of RelA$^{-/-}$ mice, which died at embryonic day 15 as a result of extensive liver apoptosis[22]. Further experiments showed that increased sensitivity to pro-apoptotic stimuli is not unique to the liver, but is also shown by RelA$^{-/-}$ fibroblasts treated with tumor necrosis factor-α (TNF-α), which—despite its reputation—is a poor inducer of apoptosis unless accompanied by inhibitors of new RNA or protein synthesis[8,10]. In cells that lack NF-κB, TNF-α can induce apoptosis on its own. Other experiments, which dissected the different signaling pathways activated by TNF-α *via* its type 1 receptor (TNFR1), provided independent evidence for the important anti-apoptotic function of NF-κB[11]. Collectively, these findings show that the products of certain NF-κB target genes inhibit apoptosis (see below). In addition, the activation of NF-κB by TNF-α attenuates the pro-apoptotic activity of the latter. Interestingly, the apoptotic signaling cascade triggered by TNF-α (**Fig. 2**)—which depends on recruitment of the Fas-associated death domain (FADD) protein to the cytoplasmic domain of TNFR1 leading to activation of caspase-8—is similar to the

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cascade triggered by another member of the TNF family, Fas ligand (FasL). Unlike TNF-α, however, FasL is a poor NF-κB activator and therefore its ability to induce apoptosis does not depend on concurrent inhibition of new gene expression[23]. By virtue of their ability to activate NF-κB and caspase-8 (or caspase-10), members of the TNF family can be regarded as either pro- or anti-apoptotic.

The suppression of apoptosis by NF-κB is an important component of TNF-α biology. The mid-gestational lethality and massive liver apoptosis of RelA$^{-/-}$ mice—which are also shown by mice that lack IKKβ or IKKγ[16,18,24]—is completely suppressed by the absence of TNFR1 or TNF-α[4,25]. The complete dependence of the fetal liver on NF-κB for its survival is due to the production of large amounts of TNF-α—even in the absence of infection—by the hematopoietic progenitors that populate the fetal liver[26]. Although it is not required for liver development *per se*, NF-κB is also important for the survival of adult liver after encounter with infectious organisms such as *Listeria monocytogenes*[27]. NF-κB activity also aids the long-term survival of lymphoid cells[28], whose demise in the absence of NF-κB can be brought about by TNF-α[7] or *via* other mechanisms (see below). In addition to the marked increase in their susceptibility to TNF-α–induced apoptosis, NF-κB–deficient epithelial cells show increased sensitivity to a variety of DNA-damaging chemotherapeutic drugs and ionizing radiation[29]. As many tumors, both of lymphoid and epithelial origin, show constitutively activated NF-κB, it is likely that the anti-apoptotic function of NF-κB represents a major obstacle to successful cancer therapy[29].

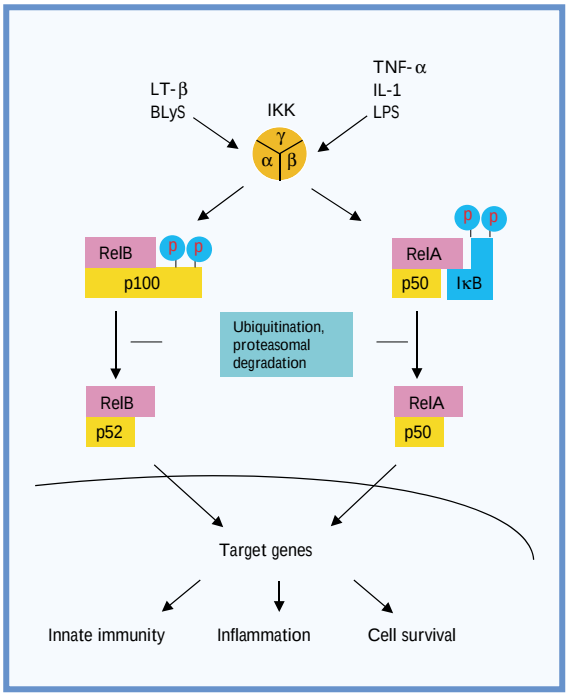

Figure 1. IKK can activate NF-κB transcription factors *via* two distinct pathways. The canonical NF-κB activation pathway—triggered by TNF-α, IL-1 or byproducts of bacterial and viral infections (such as LPS and double-stranded RNA)—is dependent on the IKKβ catalytic subunit and is accomplished through IκB phosphorylation and ubiquitin-dependent degradation. The second pathway is triggered by only a few members of the TNF family, such as lymphotoxin β (LT-β) and BLyS (also known as BAFF). This pathway is dependent on IKKα and is initiated *via* phosphorylation-dependent processing of p100. P, phosphate.

How NF-κB suppresses apoptosis

The anti-apoptotic activity of NF-κB depends on gene induction[11]. In fact, NF-κB induces the expression of a number of genes whose products can inhibit apoptosis; these include cellular inhibitors of apoptosis (c-IAPs), caspase-8–c-FLIP (FLICE inhibitory protein), A1 (also known as Bfl1), TNFR-associated factor 1 (TRAF1) and TRAF2. As TRAF1 and TRAF2 are adaptor proteins required for optimal NF-κB and c-Jun kinase (Jnk) activation, their anti-apoptotic activity is most likely due to their ability to augment the activation of NF-κB[23]. The other anti-apoptotic proteins may work in a coordinated manner to block apoptosis at multiple steps along the apoptotic signaling cascade (**Fig. 3**).

The best studied NF-κB–induced anti-apoptotic proteins are the c-IAPs, which directly bind and inhibit effector caspases, such as caspase-3 and caspase-7, as well as prevent activation of pro-caspase-6 and pro-caspase-9[30]. Thus, c-IAPs can inhibit apoptosis induced by both death receptors and mitochondria-dependent pathways. The involvement of c-Iaps in the anti-apoptotic activity of NF-κB was shown by the finding that the induction of c-*Iap2* by TNF-α or phorbol myristol acetate (PMA) + ionomycin is completely blocked in cells that stably express a degradation-resistant form (super-repressor) of IκBα[31]. Indeed, two functional κB sites were found in the *cIap2* promoter[32]. c-IAP1 expression also seems to be NF-κB–dependent[33]. The induction of c-IAP1 and c-IAP2 by NF-κB, along with TRAF1 and TRAF2, suppresses TNF-α–mediated killing through the inhibition of caspase-8[33]. Curiously, expression of c-IAP1 or c-IAP2 alone was sufficient to inhibit apoptosis induced by the DNA topoisomerase inhibitor etoposide (also a DNA-damaging agent), but did not reduce the sensitivity to TNF-α[33]. Although c-IAPs cannot bind directly to caspase-8[30], their recruitment to the TNFR1 signaling complex through an interaction with TRAF2[34] might provide sufficient proximity for inhibition[33]. c-IAPs were also suggested to function through stimulation of NF-κB activity[31]. However, this effect may be indirect and could simply be due to inhibition of caspases, which otherwise terminate NF-κB activity (see below).

Another c-IAP regulated by NF-κB is X chromosome-linked IAP (XIAP, also known as hILP)[35]. XIAP inhibits caspase-3 and caspase-7 through its second baculoviral IAP repeat (BIR) domain and NH_2-terminal linker[36] and might prevent activation of pro-caspase-9 through a region containing its third BIR domain, BIR3[37]. Structural analysis showed that the NH_2-terminal linker directly blocks the catalytic cleft of caspase-3 and caspase-7, whereas the BIR2 region facilitates caspase binding[38–40]. T cell–specific transgenic expression of XIAP in mice suppressed thymocyte apoptosis induced by various death insults[41]. However, disruption of the gene encoding XIAP in mice showed no difference in caspase-mediated apoptosis, although c-IAP1 and c-IAP2 were up-regulated[42]. This suggests the existence of a compensatory mechanism. Overexpression of XIAP can inhibit TNF-α–induced apoptosis of cells expressing the IκBα super-repressor mutant[43]. These findings indicate that XIAP may be a NF-κB target gene involved in mediating NF-κB anti-apoptotic effects. As shall be discussed below, XIAP may also function as an inhibitor of Jnk activation[44].

Another NF-κB–regulated inhibitor of apoptosis is c-FLIP, which was first identified as a cellular homolog of viral FLIP[45]. c-FLIP contains two death effector domains (DEDs) and a catalytically inactive caspase-like domain; it can interact with FADD and pro-caspase-8 through homotypic DED-mediated interactions[46]. c-FLIP may, therefore, inhibit apoptosis by interfering with pro-caspase-8 activation. Indeed, embryonic fibroblast cells from c-FLIP-deficient mice are hypersensitive to TNF-α– or FasL-induced apoptosis[47]. c-FLIP also interacts with TRAF2 and receptor-interacting protein (RIP)[48], which are responsible for activation of Jnk and IKK *via* the TNFR1 complex[23]. This raises the possibility that the anti-apoptotic effect of c-FLIP might

also be due to increases in NF-κB activation. However, TNF-α–induced NF-κB activation was unchanged in c-FLIP-deficient cells[47]. c-FLIP expression is regulated by NF-κB, as its induction by TNF-α or PMA + ionomycin is inhibited in cells that express the IκBα super-repressor[49]. Forced expression of c-FLIP in such cells prevents TNF-α–induced apoptosis[49]. Although c-FLIP is still induced by TNF-α in RelA$^{-/-}$ cells[47], NF-κB activation is required, albeit not sufficient, to induce c-FLIP[49]. It remains to be determined whether there is a κB site(s) in the c-FLIP promoter (encoded by *Cflar*), and if so, which NF-κB dimers control its expression.

NF-κB also inhibits apoptosis by DNA damaging agents, which act *via* the mitochondria-dependent pathway[29]. This activity could be mediated through members of the Bcl-2 family, such as A1 and Bcl-x_L. A1 was cloned as a hematopoietic-specific Bcl-2 homolog[50]. Its overexpression suppresses apoptosis induced by etoposide or interleukin 3 (IL-3) withdrawal and partially suppresses TNF-α–induced killing[51]. A1 also prevents mitochondrial depolarization, the release of cytochrome c and caspase-9 activation[51]. A1 may therefore exert its anti-apoptotic effects by regulating mitochondrial events. Expression of A1 is induced by several NF-κB activators, including TNF-α, PMA + ionomycin, lipopolysaccharide (LPS) antigens or overexpression of RelA, in an NF-κB–dependent manner[52–54]. Overexpressed A1 can rescue c-Rel–deficient cells[52] or IκBα super-repressor–expressing cells[54] from apoptosis induced by TNF-α or antigen receptor ligation. The *BfL* promoter (for A1) contains a functional κB site[52,54]. So far it has only been determined that A1$^{-/-}$ mice show increased neutrophil apoptosis[55], which suggests that other genes mediate the anti-apoptotic activity of NF-κB in other sites. One of these may be the gene encoding Bcl-x_L, which is induced in response to either CD28 or CD40 ligation, incubation with PMA + ionomycin or TNF-α and expression of the HTLV1 (human T cell leukemia virus 1) Tax protein[53,56–59]. Overexpression of Bcl-x_L also rescues IκBα super-repressor–expressing cells from TNF-α–induced apoptosis[59]. At this point it is not clear whether Bcl-x_L is coregulated with A1 or is used to block apoptosis in different cell types or under different circumstances. These questions should be answered through the analysis of Bcl-x_L–deficient mice. It is also possible that Bcl-2 itself may be involved in the anti-apoptotic activity of NF-κB, as its expression is defective in B cells that lack both c-Rel and RelA, the major activating subunits of NF-κB[60]. In addition, a *Bcl2* transgene partially prevents the apoptosis of such B cells engrafted to irradiated hosts[60]. However, it remains to be determined whether *Bcl2* expression is directly regulated by NF-κB.

The regulation of Bcl-2 family proteins by NF-κB also extends to the pro-apoptotic member Bax[61]. Bax expression is increased in certain cells that express the IκBα super-repressor and overexpression of NF-κB inhibits p53-stimulated *Bax* promoter activity[61]. Although there is a functional κB site that binds RelA (p50) or RelB in the *Bax* promoter, deletion of this κB site has no effect on NF-κB–mediated inhibition[61], which suggests an indirect effect. Importantly, TNF-α is unable to decrease Bax expression, as the half-life of Bax is very long. It is therefore postulated that NF-κB–mediated inhibition of Bax expression may play a role only in the survival of cancer cells that show constitutive NF-κB activity[61].

It has been reported that the putative inhibitor of apoptosis, IEX-1L, is encoded by an NF-κB target gene. Cloned by mRNA differential display as a variant of the immediate-early radiation response gene that encodes IEX-1, IEX-1L overexpression inhibits TNF-α– and FasL-induced apoptosis of Jurkat cells[62]. It was suggested that IEX-1L might be regulated by NF-κB because TNF-α failed to induce its expression in RelA$^{-/-}$ cells and its ectopic overexpression rescued IκBα super-repressor–expressing cells from TNF-α–induced killing[62]. However, it may be that IEX-1L is an artificially generated variant of IEX-1 that inhibits apoptosis by interfering with the pro-apoptotic effect of IEX-1[63]. The existence of IEX-1L certainly needs to be verified. In addition, the biochemical functions of either IEX-1 or IEX-1L are unknown.

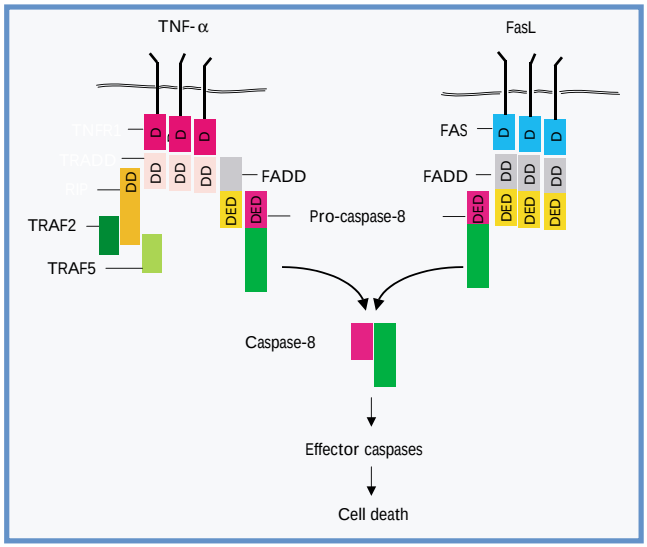

Figure 2. Signaling cascades used by TNFR1 and Fas to trigger apoptosis. The recruitment of pro-caspase-8 to Fas, TNFR1 or other death receptors upon their ligation results in its self-cleavage and activation through an induced proximity mechanism. Active caspase-8 cleaves and activates other pro-caspases. The active effector caspases cleave a variety of cellular substrates that bring about apoptotic cell death. DD, death domain; DED, death effector domain.

NF-κB can also suppress cell death by modulating the activity of anti-apoptotic and pro-apoptotic signaling pathways. Both TRAF1 and TRAF2 are NF-κB–inducible and contribute to its anti-apoptotic activity[33]. TRAF2 plays a critical role in activating the IKK and Jnk pathways that lead to NF-κB and AP-1 activation, respectively[23]. Gene-disruption experiments showed that TRAF2$^{-/-}$ cells are completely defective in TNF-α–stimulated Jnk activity, but only partially deficient in NF-κB activation[64]. Nevertheless, TRAF2$^{-/-}$ mice show increased sensitivity to TNF-α–induced apoptosis[64]. It is not clear whether the increased sensitivity to TNF-α is due to the partial reduction in NF-κB activity or the complete defect in Jnk activation.

The involvement of Jnk in TNF-α–induced apoptosis has been a highly controversial issue. Initial experiments based on overexpression of either Jnk activators or dominant-negative forms of the Jnk substrate c-Jun supported a pro-apoptotic role for Jnk in TNF-α signaling[65]. However, the dissection of TNF-α–stimulated effector pathways failed to substantiate a pro-apoptotic role for Jnk, while identifying the strong anti-apoptotic activity of NF-κB[11]. Another group who used similar experiments even suggested a weak anti-apoptotic function for Jnk alongside the strong anti-apoptotic effect of NF-κB[66]. The analysis of Jnk-deficient mouse fibroblasts failed to identify any resistance to FasL- or TNF-α–induced apoptosis[67]. Most likely, Jnk activation is not obligatory for TNF-α– or FasL-induced apoptosis, although it is important for ultraviolet-induced apoptosis, which proceeds *via* the mitochondria-dependent pathway[67]. Nevertheless, Jnk may be a positive modulator of TNF-α–induced apoptosis *via* an unknown mechanism[44]. A constitutively activated Jnk construct enhanced TNF-α–induced

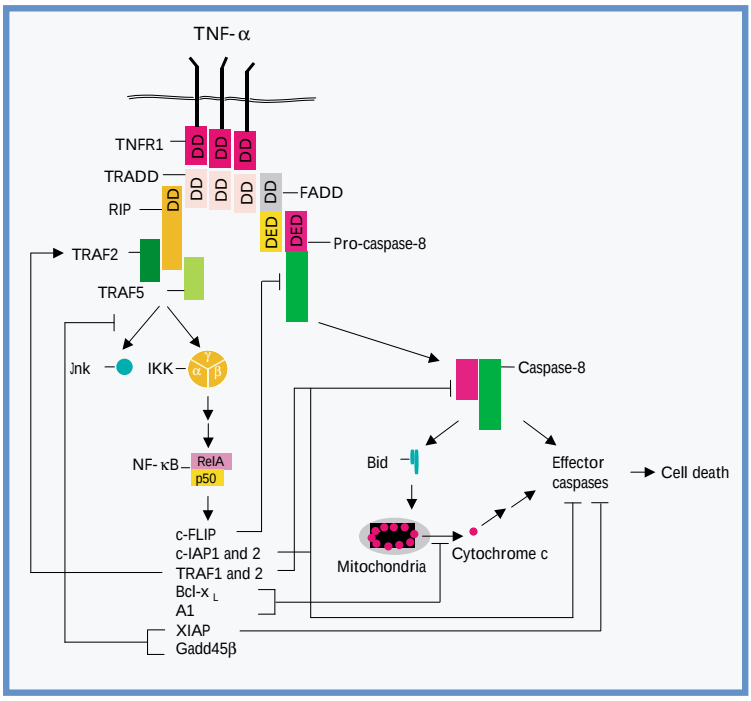

Figure 3. NF-κB induces a variety of anti-apoptotic factors that can prevent TNF-α–induced apoptosis. These anti-apoptotic factors include inhibitors of caspase activation (c-FLIP) and action (c-IAPs), anti-apoptotic Bcl2 family members (A1, Bcl-x_L) and inhibitors of Jnk activation (XIAP, GADD45β).

apoptosis of RelA$^{-/-}$ cells, whereas it had no apoptotic activity on its own[44]. In addition, Jnk activation by TNF-α was highly transient in normal cells, but was extensively prolonged in IKKβ- or RelA-deficient cells[44]. This observation led three different groups to the discovery that NF-κB can induce the expression of a Jnk inhibitor[44,68,69], which raises the possibility that this inhibitor may contribute to the anti-apoptotic function of NF-κB.

The identity of the Jnk inhibitor is a matter of debate. By examining the NF-κB–inducible anti-apoptotic factors discussed above, including cIAP1, XIAP, c-Flip, Bcl-x_L and Bcl-2, it was shown that only ectopic expression of XIAP was capable of restoring transient Jnk activation to TNF-α–treated RelA$^{-/-}$ cells[44]. XIAP had no effect on p38 or extracellular signal-regulated kinase (Erk) activation and its induction was indeed defective in RelA$^{-/-}$ cells[44]. Interestingly, the kinetics of Jnk activation by IL-1 were not altered by the loss of NF-κB activity, which suggests that XIAP or other NF-κB–induced proteins (because XIAP may not be the only inhibitor of Jnk activation induced by NF-κB) specifically interfere with signaling from TNFR1 to the Jnk cascade. A different and elegant approach was undertaken when a cDNA library from TNF-α–treated wild-type mouse fibroblasts was screened for genes capable of preventing TNF-α–induced apoptosis of RelA$^{-/-}$ cells[68]. In addition to identifying known inhibitors of TNF-α–induced cell killing, including RelA and c-FLIP, this screen netted a cDNA encoding GADD45β[68]. Like other members of its family, expression of the GADD45β protein is induced in response to growth inhibition or DNA damage[70]. *Gadd45b* is the only gene in this family that appears to be regulated by NF-κB, and most importantly its ectopic expression in RelA$^{-/-}$ fibroblasts completely suppresses TNF-α–induced apoptosis[68]. Curiously, the anti-apoptotic activity of GADD45β extends to the mitochondria-dependent pathway, as it also blocks cell death induced by DNA-damaging agents[68]. Expression of GADD45β also abrogated the prolonged Jnk activation response in TNF-α–treated RelA$^{-/-}$ cells[68]. In addition, it has also been suggested that the anti-apoptotic activity of GADD45β may be due to its ability to inhibit Jnk activation[68]. This suggestion, however, is inconsistent with most of the findings discussed above, according to which Jnk is not a critical mediator of death receptor-induced apoptosis. In addition, Gadd45β$^{-/-}$ mice are viable and do not show the severe liver apoptosis associated with loss of NF-κB activity (A. Fornace and D. Liebermann, personal communication).

A possible explanation for some of the results obtained[44,68] is that GADD45β and XIAP are not directly involved in the regulation of Jnk activity. It has been suggested that caspase activation by death receptors may result in prolonged Jnk activation[71]. Thus, any factor that inhibits TNF-α–induced apoptosis may obliterate the delayed phase of the Jnk activation response seen in TNF-α–treated RelA$^{-/-}$ cells. Nevertheless, XIAP may be a genuine inhibitor of Jnk activation, as it was capable of inhibiting Jnk activation in HeLa cells, which, unlike RelA$^{-/-}$ fibroblasts, do not undergo apoptosis after challenge with TNF-α alone[44].

The take-home lesson from the results reviewed above is that NF-κB activates multiple target genes whose products can block the apoptotic program triggered by either death receptors or the mitochondrial pathway. The relative importance of these factors may depend on the cell type and the particular situation in which they are examined. Strangely, none of the mouse strains analyzed so far that lack a single NF-κB–inducible anti-apoptotic factor have shown the extensive liver apoptosis associated with loss of RelA or IKKβ. Either multiple NF-κB–induced factors are involved in the suppression of TNF-α–induced apoptosis or the critical factor is yet to be identified.

Pro-apoptotic stimuli and NF-κB activation

If NF-κB–dependent gene expression is such a major roadblock on the path to apoptosis, then pro-apoptotic stimuli must find ways to circumvent it. Indeed, several key components in the NF-κB activation pathway seem to be targeted by caspases, which results in the termination of its anti-apoptotic activity.

At least two of the signaling proteins involved in TNF-α–induced NF-κB activation, RIP and TRAF2, are caspase substrates. Caspase-8 cleaves RIP at Asp^{32} (ref. 72). This proteolysis, which produces an NH_2-terminal truncated fragment (RIPc) and a COOH-terminal–truncated fragment (RIPn), eliminates the ability of RIP to signal to IKK[72]. Overexpression of RIPc enhances the association between TNFR1, TRADD and FADD, while inhibiting NF-κB activation, thereby promoting cell killing by TNF-α[72]. TRAF2 is also proteolyzed or sequestered into a nonsoluble cellular compartment upon recruitment to CD30 (a member of the TNFR family), which results in increased sensitivity to TNF-α[73,74]. This down-regulation of TRAF2 appears to be specific to CD30 ligation[75,76]. Proteolysis of TRAF1 may also contribute to turning off of the anti-apoptotic activity of NF-κB[76,77]. TRAF1 is cleaved at Asp^{163} by caspase-8 during TNFR1- or Fas-induced apoptosis, which generates two fragments[76,77]. Expression of the TRAF1 COOH-terminal fragment inhibits NF-κB activation by cotransfected TRAF2 or TNFR1 and promotes TNF-α– and Fas-mediated killing[76,77]. As the COOH-terminal fragment contains the TRAF domain, it may disrupt the interaction of TRAF1 with TRAF2, as well as with c-IAP1 and c-IAP2[77] by acting as a dominant-negative isoform.

The IKKβ catalytic subunit of the IKK complex is essential for NF-κB activation by TNF-α[18]. IKKβ, but not IKKα or IKKγ, is proteolyzed by caspase-3–related caspases during TNF-α– or Fas-induced apoptosis[78]. Cleavage of IKKβ occurs at Asp[78], Asp[214], Asp[373] and Asp[546] and results in the elimination of its enzymatic activity[78]. One of the fragments, IKKβ(1–546) appears to act as an inhibitor of IKK and can promote TNF-α–induced apoptosis upon overexpression[78]. Most importantly, overexpression of a caspase-resistant form of IKKβ can prevent TNF-α–induced apoptosis[78]. The proteolysis of IKKβ may therefore be a critical event in the decision between life and death. In wild-type cells, IKK was activated repeatedly in response to TNF-α, but eventually its activation was blunted as IKKβ was proteolyzed. In contrast, in cells expressing the caspase-resistant IKKβ mutant, IKK was repeatedly activated for a long time[78].

Ubiquitination-mediated proteolysis of IκBα by the 26*S* proteosome is a critical step in NF-κB activation[12]. Caspase-3–mediated cleavage can prevent IκBα degradation by separating its regulatory NH_2-terminal domain (which contains the IKK phosphorylation sites) from the body of the inhibitor. This generates a super-repressor–like molecule that is resistant to TNF-α–induced phosphorylation and degradation[79]. Phosphorylation of IκBα by IKK or substitution of Ser[32] and Ser[36] with glutamates to mimic their phosphorylation blocked proteolysis by caspases[80]. Thus, IκBα may be another important component in the life or death switch.

NF-κB itself is also on the caspase hit-list. RelA is cleaved during apoptosis induced by growth factor withdrawal at its COOH-terminal activation domain by several caspases[81]. This generates RelA with a truncated COOH-terminal that can bind DNA but is unable to activate transcription[81]. Thus, the proteolysis of RelA converted NF-κB into its own inhibitor, while expression of a caspase-resistant RelA mutant can protect cells against death caused by growth factor withdrawal[81].

To efficiently eliminate the protection conveyed by the IKK–NF-κB survival pathway, apoptotic cells also use caspases to directly cleave NF-κB–induced anti-apoptotic gene products. For instance, c-IAP1 is cleaved by caspase-3–related caspases and overexpression of c-IAP1 proteolytic fragments induces apoptosis[82]. Likewise, cleavage of XIAP by caspases at Asp[24] resulted in two proteolytic fragments: the NH_2-terminal fragment weakly inhibits caspase-3 and caspase-7, whereas the COOH-terminal fragment is capable of potentiating Fas-induced apoptosis[37]. Bcl-x_L is cleaved by caspases during apoptosis induced by cytokine withdrawal, viral infection[83,84] or by a different protease, called calpain, during apoptosis caused by ischemic injury or exposure to amyloid β peptide[85]. Proteolytic cleavage converts Bcl-x_L into two pro-apoptotic fragments[83,84]. However, it is yet to be determined whether Bcl-x_L is proteolyzed during TNF-α–induced apoptosis and whether the cleavage-resistant Bcl-x_L (D61A) mutant can suppress cell killing by TNF-α.

In addition to these caspase-dependent mechanisms, the ingestion of apoptotic bodies by macrophages seems to turn off the induction of NF-κB–dependent target genes[86]. Although the mechanism that accounts for this phenomenon remains to be explored, the uptake of apoptotic bodies down-regulates the production of TNF-α and other inflammatory cytokines and instead switches on the expression of anti-inflammatory cytokines such as transforming growth factor-β (TGF-β). This response is likely triggered through the binding of phosphatidylserine (PS)—which is present on the outside of apoptotic bodies—to the PS receptor (PSR)[87]. The parasite *Trypanosoma cruzi* takes advantage of this phenomenon by inducing extensive lymphocyte apoptosis, which suppresses the inflammatory response of macrophages that ingest the apoptotic lymphocytes. This provides a hospitable environment that promotes the replication of *T. cruzi* within macrophages[88]. A different mechanism that also leads to inhibition of NF-κB activation is used by *Yersinia pestis*, the agent that causes bubonic plague. The *Y. pestis* virulence factor, YopJ, is an ubiquitin-like protein protease that binds to mitogen-activated protein kinase (MAPK) kinase kinases (MAP3Ks) as well as IKK and inhibits their activation[89]. This strategy not only allows *Y. pestis* to subdue the host inflammatory response, but likely contributes to its extreme virulence and ability to induce the apoptosis of host cells[90].

Although the majority of evidence—especially that generated by the analysis of knockout mice—provides overwhelmingly strong support to the anti-apoptotic function of NF-κB, there are a few sporadic reports that NF-κB may contribute to induction of pro-apoptotic molecules. This list includes death receptor 6 (DR6)—a member of the TNFR family[91]—DR4, DR5[92] and Fas[93]. However, in each case when NF-κB–induced expression of these death receptors was detected, so was the induction of anti-apoptotic molecules that neutralized their killing activity. Thus, as yet, little physiological evidence exists for a pro-apoptotic function of NF-κB.

Anti-apoptosis and immune regulation by NF-κB

Because of its ability to suppress apoptosis, NF-κB likely plays a central role in the regulation of both innate and adaptive immune responses. Although the specific elimination of virally and bacterially infected cells *via* apoptosis may seem to be an effective defense strategy, sealing the contents of such cells within highly condensed apoptotic bodies is an anti-inflammatory mechanism that prevents the activation of innate and adaptive immune responses. In addition to interfering with antigen presentation, the formation of an apoptotic body prevents the leakage of normal cellular constituents from infected or injured cells. The release of normal cytoplasmic proteins, such as heat-shock proteins, can result in the activation of Toll-like receptors (TLRs) and the triggering of innate immune responses[94,95]. TLR activation also stimulates the maturation of dendritic cells, thereby increasing the efficacy of antigen presentation as well as inducing the production of various cytokines that further stimulate adaptive immunity[96]

The production of proinflammatory cytokines by dendritic cells and other types of antigen-presenting cells (APCs) is of particular importance in preventing the apoptosis of T cells that have not received proper costimulation. It is well established that T cell receptor (TCR) ligation in the absence of costimulation can result in T cell apoptosis. The need for costimulation (such as ligation of CD28 on T cells by its ligand B7 on B cells) can be bypassed with the use of adjuvants, which have long been known for their ability to potentiate the production of antibodies to T cell–dependent antigens. All adjuvants are potent TLR agonists and therefore activators of NF-κB[94]. TCR stimulation *in vivo* (by antigen injection) in the presence of adjuvants results in the induction within T cells (which do no undergo apoptosis under these conditions) of RelB and the IκB-like protein Bcl-3[97]. Unlike the conventional IκBs, Bcl-3 binds to p50 or p52 homodimers in the nucleus and acts as a coactivator that converts them from repressors of NF-κB target genes to activators[98]. Thus, its induction in T cells is likely to stimulate the expression of NF-κB target genes. Indeed, the introduction of ectopic Bcl-3 to T cells prevents the induction of apoptosis by TCR ligation in the absence of costimulation or adjuvants[97].

Whereas costimulation acts directly on the T cell to potentiate IKK activation by TCR ligation, T cells do not express TLRs. Most likely, the anti-apoptotic effects of adjuvants in this system are exerted *via* TLR activation on dendritic cells. Another way in which the anti-apoptotic

activity of NF-κB can promote adaptive immune responses is by contributing to the maturation of B cells, which is required for production of antibodies to T cell–dependent antigens[15]. As B cells mature, they become less sensitive to apoptosis. This process depends on the IKKα subunit of IKK, which specifically promotes the activation of p52-RelB dimers. It remains to be determined whether these dimers activate anti-apoptotic genes other than those induced by the p50-RclA dimers.

Regardless of the mechanism by which it is achieved, these examples clearly illustrate how the anti-apoptotic activity of NF-κB is intimately linked to its ability to stimulate and orchestrate innate and adaptive host defenses. As discussed above, the ability of NF-κB to suppress apoptosis and promote inflammation is likely mediated *via* a large number of target genes. One should not forget, however, that NF-κB transcription factors do not work alone. Many NF-κB target genes are also responsive to other transcription factors, including AP-1 and C/EBP-β[2]. As the activities of such transcription factors are regulated by other signaling pathways, such as the MAPKs, the combinational nature of transcriptional control provides a means for modulating the repertoire of NF-κB target genes that are induced in response to a given stimulus. Such modulation likely plays a key role in determining the exact nature of immune responses and host defenses elicited by different pathogens.

In conclusion, NF-κB is a central regulator of innate and adaptive immune responses. This function is accomplished through the induction of genes, some of which promote inflammation, leukocyte migration and activation, whereas others act as potent inhibitors of apoptosis. It may be desirable under certain circumstances, such as during cancer therapy, to dissociate the immunoregulatory function of NF-κB from its anti-apoptotic activity. However, this may not be easily achieved without a better understanding of all the mechanisms involved in the activation of specific and physiologically relevant NF-κB target genes. The complete understanding of NF-κB–dependent gene regulation is therefore a major challenge for future research.

Acknowledgments

We thank C. Adams for help with manuscript preparation and A. Fornace for disclosing unpublished results. Supported by grants from the National Institutes of Health, the State of California Cancer Research Program and the American Cancer Society.

1. Baeuerle, P. A. & Henkel, T. Function and activation of NF-κB in the immune system. *Annu. Rev. Immunol.* **12**, 141–179 (1994).
2. Barnes, P. J. & Karin, M. NF-κB – A pivotal transcription factor in chronic inflammatory diseases. *New Engl. J. Med.* **336**, 1066–1071 (1997).
3. Sha, W. C., Liou, H. C., Tuomanen, E. I. & Baltimore, D. Targeted disruption of the p50 subunit of NF-κB leads to multifocal defects in immune responses. *Cell* **80**, 321–330 (1995).
4. Alcamo, E. *et al.* Targeted mutation of TNF receptor I rescues the RelA-deficient mouse and reveals a critical role for NF-κB in leukocyte recruitment. *J. Immunol.* **167**, 1592–1600 (2001).
5. Franzoso, G. *et al.* Mice deficient in NF-κB/p52 present with defects in humoral responses, germinal center reactions, and splenic reactions. *J. Exp. Med.* **187**, 147–159 (1998).
6. Attar, R. M. *et al.* Genetic approaches to study Rel/NF-κB/IκB function in mice. *Semin. Cancer Biol.* **8**, 93–101 (1997).
7. Senftleben, U., Li, Z.-W., Baud, V. & Karin, M. IKKβ is essential for protecting T cells from TNFα-induced apoptosis. *Immunity* **14**, 217–230 (2001).
8. Beg, A. A. & Baltimore, D. An essential role for NF-κB in preventing TNF-α induced cell death. *Science* **274**, 782–784 (1996).
9. Wang, C.-Y., Mayo, M. W. & Baldwin, A. S. Jr TNF- and cancer therapy-induced apoptosis: potentiation by inhibition of NF-κB. *Science* **274**, 784–787 (1996).
10. Van Antwerp, D. J., Martin, S. J., Kafri, T., Green, D. R. & Verma, I. M. Suppression of TNFα-induced apoptosis by NF-κB. *Science* **274**, 787–789 (1996).
11. Liu, Z.-G., Hu, H., Goeddel, D.V. & Karin, M. Dissection of TNF receptor 1 effector functions: JNK activation is not linked to apoptosis, while NF-κB activation prevents cell death. *Cell* **87**, 565–576 (1996).
12. Karin, M. & Ben-Neriah, Y. Phosphorylation meets ubiquitination: the control of NF-κB activity. *Annu. Rev. Immunol.* **18**, 621–663 (2000).
13. Ghosh, S., May, M. J. & Kopp, E. B. NF-κB and Rel proteins: evolutionarily conserved mediators of immune responses. *Annu. Rev. Immunol.* **16**, 225–260 (1998).
14. Solan, N. J., Miyoshi, H., Bren, G. D. & Paya, C.V. RelB cellular regulation and transcriptional activity are regulated by p100. *J. Biol. Chem.* **277**, 1405–1418 (2002).
15. Senftleben, U. *et al.* Activation by IKKα of a second, evolutionary conserved, NF-κB signaling pathway. *Science* **293**, 1495–1499 (2001).
16. Li, Q., Van Antwerp, D., Mercurio, F., Lee, K.-F. & Verma, I. M. Severe liver degeneration in mice lacking the IκB kinase 2 gene. *Science* **284**, 321–325 (1999).
17. Delhase, M., Hayakawa, M., Chen, Y. & Karin, M. Positive and negative regulation of IκB kinase activity through IKKβ subunit phosphorylation. *Science* **284**, 309–313 (1999).
18. Li, Z.-W. *et al.* The IKKβ subunit of IκB kinase (IKK) is essential for NF-κB activation and prevention of apoptosis. *J. Exp. Med.* **189**, 1839–1845 (1999).
19. Zhong, H., SuYang, H., Erdjument-Bromage, H., Tempst, P. & Ghosh, S. The transcriptional activity of NF-κB is regulated by the IκB-associated PKAc subunit through a cyclic AMP-independent mechanism. *Cell* **89**, 413–424 (1997).
20. Sizemore, N., Leung, S. & Stark, G. R. Activation of phosphatidylinositol 3-kinase in response to Interleukin-1 leads to phosphorylation and activation of the NF-κB p65/RelA subunit. *Mol. Cell. Biol.* **19**, 4798–4805 (1999).
21. Madrid, L.V. *et al.* Akt suppresses apoptosis by stimulating the transactivation potential of the RelA/p65 subunit of NF-κB. *Mol. Cell. Biol.* **20**, 1626–1638 (2000).
22. Beg, A. A., Sha, W. C., Bronson, R. T., Ghosh, S. & Baltimore, D. Embryonic lethality and liver degeneration in mice lacking the RelA component of NF-κB. *Nature* **376**, 167–169 (1995).
23. Baud, V. & Karin, M. Signal transduction by tumor necrosis factor and its relatives. *Trends Cell. Biol.* **11**, 372–377 (2001).
24. Makris, C. *et al.* Female mice heterozygote for IKKγ/NEMO deficiencies develop a genodermatosis similar to the human X-linked disorder Incontinentia Pigmenti. *Mol. Cell* **15**, 969–979 (2000).
25. Doi, T. S. *et al.* Absence of TNF rescues RelA-deficient mice from embryonic lethality. *Proc. Natl Acad. Sci. USA* **96**, 2994–2999 (1999).
26. Rosenfeld, M. E., Prichard, L., Shiojiri, N. & Fausto, N. Prevention of hepatic apoptosis and embryonic lethality in RelA/TNFR1 double knockout mice. *Am. J. Pathol.* **156**, 997–1007 (2000).
27. Lavon, I. *et al.* High susceptibility to bacterial infection, but no liver dysfunction, in mice compromised for hepatocyte NF-κB activation. *Nature Med.* **6**, 573–577 (2000).
28. Horwitz, B. H., Scott, M. L., Cherry, S. R., Bronson, R. T. & Baltimore, D. Failure of lymphopoiesis after adoptive transfer of NF-κB-deficient fetal liver cells. *Immunity* **6**, 765–772 (1997).
29. Baldwin, A. S. Control of oncogenesis and cancer therapy resistance by the transcription factor NF-κB. *J. Clin. Invest.* **107**, 241–246 (2001).
30. Deveraux, Q. L. *et al.* IAPs block apoptotic events induced by caspase-8 and cytochrome c by direct inhibition of distinct caspases. *EMBO J.* **17**, 2215–2223 (1998).
31. Chu, Z. L. *et al.* Suppression of TNF-induced cell death by inhibitor of apoptosis c-IAP2 is under NF-κB control. *Proc. Natl Acad. Sci. USA* **94**, 10057–10062 (1997).
32. Hong, S.Y. *et al.* Involvement of two NF-κB binding elements in TNFα-, CD40-, and Epstein-Barr virus latent membrane protein 1-mediated induction of the cellular inhibitor of apoptosis protein 2 gene. *J. Biol. Chem.* **275**, 18022–18028 (2000).
33. Wang, C. Y., Mayo, M. W., Korneluk, R. G., Goeddel, D.V. & Baldwin, A. S. Jr NF-κB antiapoptosis: induction of TRAF1 and TRAF2 and c-IAP1 and c-IAP2 to suppress caspase-8 activation. *Science* **281**, 1680–1683 (1998).
34. Shu, H. B., Takeuchi, M. & Goeddel, D.V. The TNF2 signal transducers TRAF2 and c-IAP1 are components of the TNF1 signaling complex. *Proc. Natl Acad. Sci. USA* **93**, 13973–13978 (1996).
35. Liston, P. *et al.* Suppression of apoptosis in mammalian cells by NAIP and a related family of IAP genes. *Nature* **379**, 349–353 (1996).
36. Takahashi, R. *et al.* A single BIR domain of XIAP sufficient for inhibiting caspases. *J. Biol. Chem.* **273**, 7787–7790 (1998).
37. Deveraux, Q. L. *et al.* Cleavage of human inhibitor of apoptosis protein XIAP results in fragments with distinct specificities for caspases. *EMBO J.* **18**, 5242–5251 (1999).
38. Chai, J. *et al.* Structural basis of caspase-7 inhibition by XIAP. *Cell* **104**, 769–780 (2001).
39. Huang, Y. *et al.* Structural basis of caspase inhibition by XIAP: differential roles of the linker *versus* the IBR domain. *Cell* **104**, 781–790 (2001).
40. Riedl, S. J. *et al.* Structural basis for the inhibition of caspase-3 by XIAP. *Cell* **104**, 791–800 (2001).
41. Conte, D., Liston, P., Wong, J. W., Wight, K. E. & Korneluk, R. G. Thymocyte-targeted overexpression of xiap transgene disrupts T lymphoid apoptosis and maturation. *Proc. Natl Acad. Sci. USA* **98**, 5049–5054 (2001).
42. Harlin, H., Reffey, S. B., Duckett, C. S., Lindsten, T. & Thompson, C. B. Characterization of XIAP-deficient mice. *Mol. Cell. Biol.* **21**, 3604–3608 (2001).
43. Stehlik, C. *et al.* NF-κB-regulated xiap gene expression protects endothelial cells from TNFα-induced apoptosis. *J. Exp. Med.* **188**, 211–216 (1998).
44. Tang, G. *et al.* Inhibition of JNK activation through NF-κB target genes. *Nature* **414**, 313–317 (2001).
45. Thome, M. *et al.* Viral FLICE-inhibitory proteins (FLIPs) prevent apoptosis induced by death receptors. *Nature* **386**, 517–521 (1997).
46. Irmler, M. *et al.* Inhibition of death receptor signals by cellular FLIP. *Nature* **388**, 190–195 (1997).
47. Yeh, W. C. *et al.* Requirement for Casper (c-FLIP) in regulation of death receptor-induced apoptosis and embryonic development. *Immunity* **12**, 633–642 (2000).
48. Shu, H. B., Halpin, D. R. & Goeddel, D.V. Casper is a FADD- and caspase-related inducer of apoptosis. *Immunity* **6**, 751–763 (1997).
49. Kreuz, S., Siegmund, D., Scheurich, P. & Wajant, H. NF-κB inducers upregulate cFLIP, a cycloheximide-sensitive inhibitor of death receptor signaling. *Mol. Cell. Biol.* **21**, 3964–3973 (2001).
50. Lin, E.Y., Orlofsky, A., Berger, M. S. & Prystowsky, M. B. Characterizaiton of A1, a novel hemopoietic-specific early-response gene with sequence similarity to bcl-2. *J. Immunol.* **151**, 1979–1988 (1993).
51. Wang, C. Y., Guttridge, D. C., Mayo, M. W. & Baldwin, A. S. Jr NF-κB induces expression of the Bcl-2 homologue A1/Bfl-1 to preferentially suppress chemotherapy-induced apoptosis. *Mol. Cell. Biol.* **19**, 5923–5929 (1999).
52. Grumont, R. J., Rourke, I. J. & Gerondakis, S. Rel-dependent induction of A1 transcription is required to protect B cells from antigen receptor ligation-induced apoptosis. *Genes Dev.* **13**, 400–411 (1998).
53. Lee, H. H., Dadgostart, H., Cheng, Q., Shu, J. & Cheng, G. NF-κB-mediated upregulation of Bcl-X and Bfl-1/A1 is required for CD40 survival signaling in B lymphocytes. *Proc. Natl Acad. Sci. USA* **96**, 9136–9141 (1999).
54. Zong, W. X., Edelstein, L. C., Chen, C., Bash, J. & Gelinas, C. The prosurvival Bcl-2 homolog Bfl-1/A1 is a direct transcriptional target of NF-κB that blocks TNFα-induced apoptosis. *Genes Dev.* **13**, '–' (1999).
55. Hamasaki, A. *et al.* Accelerated neutrophil apoptosis in mice lacking A1-a, a subtype of the bcl-2-related A1 gene. *J. Exp. Med.* **188**, 1985–1992 (1998).
56. Tamatani, M. *et al.* TNF induces Bcl-2 and Bcl-x expression through NF-κB activation in primary hippocampal neurons. *J. Biol. Chem.* **274**, 8531–8538 (1999).
57. Tsukahara, T. *et al.* Induction of Bcl-x(L) expression by human T-cell leukemia virus type 1 Tax through NF-κB in apoptosis-resistant T-cell transfectants with Tax. *J. Virol.* **73**, 7981–7987 (1999).
58. Khoshnan, A. *et al.* The NF-κB cascade is important in Bcl-xL expression and for the anti-apoptotic effects of the CD28 receptor in primary human $CD4^+$ lymphocytes. *J. Immunol.* **165**, 1743–1754 (2000).
59. Chen, C., Edelstein, L. C. & Gelinas, C. The Rel/NF-κB family directly activates expression of the apoptosis inhibitor Bcl-x(L). *Mol. Cell. Biol.* **20**, 2687–2695 (2000).
60. Grossmann, M. *et al.* The anti-apoptotic activities of Rel and RelA required during B-cell maturation involve the regulation of Bcl-2 expression. *EMBO J.* **19**, 6351–6360 (2000).
61. Bentires-Alj, M. *et al.* Inhibition of the NF-κB transcription factor increases Bax expression in cancer

cell lines. *Oncogene* **20**, 2805–2813 (2001).
62. Wu, M. X., Ao, Z., Prasad, K. V., Wu, R. & Schlossman, S. F. IEX-1L, an apoptosis inhibitor involved in NF-κB-mediated cell survival. *Science* **281**, 998–1001 (1998).
63. Schafer, H., Arlt, A., Trauzold, A., Hunermann-Jansen, A. & Schmidt, W. E. The putative apoptosis inhibitor IEX-1L is a mutant nonspliced variant of p22(PRG1/IEX-1) and is not expressed *in vivo*. *Biochem. Biophys. Res. Commun.* **262**, 139–145 (1999).
64. Lee, S. Y. *et al.* TRAF2 is essential for JNK but not NF-κB activation and regulates lymphocyte proliferation and survival. *Immunity* **7**, 703–713 (1997).
65. Verheij, M. *et al.* Requirement for ceramide-initiated SAPK/JNK signalling in stress-induced apoptosis. *Nature* **380**, 75–79 (1996).
66. Natoli, G. *et al.* Activation of SAPK/JNK by TNFR1 through a noncytotoxic TRAF2-dependent pathway. *Science* **275**, 200–203 (1997).
67. Tournier, C. *et al.* Requirement of JNK for stress-induced activation of the cytochrome c-mediated death pathway. *Science* **288**, 870–874 (2000).
68. De Smaele, E. *et al.* Induction of gadd45β by NF-κB downregulates pro-apoptotic JNK signalling. *Nature* **414**, 308–313 (2001).
69. Javelaud, D. & Besancon, F. NF-κB activation results in rapid inactivation of JNK in TNFα-treated Ewing sarcoma cells: a mechanism for the anti-apoptotic effect of NF-κB. *Oncogene* **20**, 4365–4372 (2001).
70. Fornace, A. J. J., Jackman, J., Hollander, M. C., Hoffman-Liebermann, B. & Liebermann, D. A. Genotoxic-stress-response genes and growth-arrest genes. gadd, MyD, and other genes induced by treatments eliciting growth arrest. *Ann. NY Acad. Sci.* **663**, 139–153 (1992).
71. Lenczowski, J. M. *et al.* Lack of a role for Jun kinase and AP-1 in Fas-induced apoptosis. *Mol. Cell. Biol.* **17**, 170–181 (1997).
72. Lin, D. *et al.* A mammalian PAR-3-PAR-6 complex implicated in Cdc42/Rac1 and aPKC signalling and cell polarity. *Nature Cell Biol.* **2**, 540–547 (2000).
73. Duckett, C. S. & Thompson, C. B. CD30-dependent degradation of TRAF2: implications for negative regulation of TRAF signaling and the control of cell survival. *Genes Dev.* **11**, 2810–2821 (1997).
74. Arch, R. H., Gedrich, R. W. & Thompson, C. B. Translocation of TRAF proteins regulates apoptotic threshold of cells. *Biochem. Biophys. Res. Commun.* **272**, 936–45 (2000).
75. Lin, Y., Devin, A., Rodriguez, Y. & Liu, Z. G. Cleavage of the death domain kinase RIP by caspase-8 prompts TNF-induced apoptosis. *Genes Dev.* **13**, 2514–2526 (1999).
76. Leo, E. *et al.* TRAF1 is a substrate of caspases activated during TNFRα-induced apoptosis. *J. Biol. Chem.* **276**, 8087–8093 (2001).
77. Schwenzer, R. *et al.* The human TNF TRAF1 is upregulated by cytokines of the TNF ligand family and modulates TNF-induced activation of NF-κB and c-Jun N-terminal kinase. *J. Biol. Chem.* **274**, 19368–19374 (1999).
78. Tang, G., Yang, J., Minemoto, Y. & Lin, A. Blocking caspase-3-mediated proteolysis of IKKβ suppresses TNFα-induced apoptosis. *Mol. Cell* **8**, 1005–1016 (2001).
79. Reuther, J. Y. & Baldwin, A. S. Jr Apoptosis promotes a caspase-induced amino-terminal truncation of IκBα that functions as a stable inhibitor of NF-κB. *J. Biol. Chem.* **274**, 20664–20670 (1999).
80. Barkett, M., Xue, D., Horvitz, H. R. & Gilmore, T. D. Phosphorylation of IκBα inhibits its cleavage by caspase CPP32 *in vitro*. *J. Biol. Chem.* **272**, 29419–29422 (1997).
81. Levkau, B., Scatena, M., Giachelli, C. M., Ross, R. & Raines, E. W. Apoptosis overrides survival signals through a caspase-mediated dominant-negative NF-κB loop. *Nature Cell Biol.* **1**, 227–233 (1999).
82. Clem, R. J. *et al.* c-IAP1 is cleaved by caspases to produce a proapoptotic C-terminal fragment. *J. Biol. Chem.* **276**, 7602–7608 (2001).
83. Clem, R. J. *et al.* Modulation of cell death by Bcl-XL through caspase interaction. *Proc. Natl Acad. Sci. USA* **95**, 554–559 (1998).
84. Fujita, N., Nagahashi, A., Nagashima, K., Rokudai, S. & Tsuruo, T. Acceleration of apoptotic cell death after the cleavage of Bel-XL protein by caspase-3-like proteases. *Oncogene* **17**, 1295–1304 (1998).
85. Nakagawa, T. & Yuan, J. Cross-talk between two cysteine protease families: activation of caspase-12 by calpain in apoptosis. *J. Cell Biol.* **150**, 887–894 (2000).
86. Fadok, V. A. *et al.* Macrophages that have ingested apoptotic cells *in vitro* inhibit proinflammatory cytokine production through autocrine/paracrine mechanisms involving TGFβ, PGE2, and PAF. *J. Clin. Invest.* **101**, 890–898 (1998).
87. Fadok, V. A. *et al.* A receptor for phosphatidylserine-specific clearance of apoptotic cells. *Nature* **405**, 85–90 (2000).
88. Freire-de-Lima, C. G. *et al.* Uptake of apoptotic cells drives the growth of a pathogenic trypanosome in macrophages. *Nature* **403**, 199–203 (2000).
89. Orth, K. *et al.* Disruption of signaling by Yersinia effector YopJ, a ubiquitin-like protein protease. *Science* **290**, 1594–1597 (2000).
90. Mills, S. D. *et al.* *Yersinia enterocolitica* induces apoptosis in macrophages by a process requiring functional type III secretion and translocation mechanisms and involving YopP, presumably acting as an effector protein. *Proc. Natl Acad. Sci. USA* **94**, 12638–12643 (1997).
91. Kasof, G. M. *et al.* TNFα induces the expression of DR6, a member of the TNF receptor family, through activation of NF-κB. *Oncogene* **20**, 7965–7975 (2001).
92. Ravi, R. *et al.* Regulation of death receptor expression and TRAIL/Apo2L-induced apoptosis by NF-κB. *Nature Cell Biol.* **3**, 409–416 (2001).
93. Zheng, Y. *et al.* NF-κB RelA (p65) is essential for TNFα-induced fas expression but dispensable for both TCR-induced expression and activation-induced cell death. *J. Immunol.* **166**, 4949–4957 (2001).
94. Asea, A. *et al.* HSP70 stimulates cytokine production through a CD14-dependant pathway, demonstrating its dual role as a chaperone and cytokine. *Nature Med.* **6**, 435–442 (2000).
95. Ohashi, K., Burkart, V., Flohe, S. & Kolb, H. Cutting edge: heat shock protein 60 is a putative endogenous ligand of the toll-like receptor-4 complex. *J. Immunol.* **164**, 558–561 (2000).
96. Medzhitov, R. CpG DNA: security code for host defense. *Nature Immunol.* **2**, 15–16 (2001).
97. Mitchell, T. C. *et al.* Immunological adjuvants promote activated T cell survival *via* induction of Bcl-3. *Nature Immunol.* **2**, 397–402 (2001).
98. Dechend, R. *et al.* The Bcl-3 oncoprotein acts as a bridging factor between NF-κB/Rel and nuclear co-regulators. *Oncogene* **18**, 3316–3323 (1999).