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In the Crosshairs: NF-κB Targets the JNK Signaling Cascade

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Abstract

NF-κB/Rel transcription factors are well-known for their roles in the regulation of inflammation and immunity. NF-κB also blocks programmed cell death (PCD) or apoptosis triggered by proinflammatory cytokine, tumor necrosis factor (TNF)α. Through transcriptional induction of distinct subsets of cyto-protective target genes, NF-κB inhibits the execution of apoptosis activated by this cytokine. This protective action is mediated, in part, by factors (such as A20, GADD45β, and XIAP) that downregulate the pro-apoptotic c-Jun-N-terminal (JNK) pathway. A suppression of reactive oxygen species (ROS), which are themselves major cell death-inducing elements activated by TNFα, is an additional protective function recently ascribed to NF-κB. This function of NF-κB involves an induction of mitochondrial anti-oxidant enzyme, manganese superoxide dismutase (Mn-SOD), and a control of cellular iron availability through upregulation of Ferritin heavy chain – one of two subunits of Ferritin, the major iron storage protein complex of the cell. An emerging view of NF-κB is that, while integrated, its actions in immunity and in promoting cell survival are executed through upregulation of distinct subsets of target genes. Thus, these inducible blockers of apoptosis may provide potential new targets to inhibit specific functions of NF-κB. In the future, this might allow for a better treatment of complex human diseases involving dysregulated NF-κB activity, including chronic inflammatory conditions and cancer.

Keywords

Apoptosis; Ferritin; Gadd45β; inflammation; JNK; NF-κB; TNFα; reactive oxygen species (ROS)

INTRODUCTION

Programmed cell death (PCD) - often executed in the form of apoptosis - is a physiological process that cells use to actively undergo self-destruction [1]. In multicellular organisms, PCD contributes not only to organogenesis during embryonic development, but also to tissue homeostasis in adult organs. In fact, PCD also represents a way of eliminating abnormal cells, such as infected or cancerous cells. Accordingly, dysregulation of PCD in tissues can lead to serious complications on overall organismal physiology. In humans, defects in apoptosis have been linked to pathological conditions such as cancer, neurodegenerative and autoimmune diseases [2,3,4].

Nuclear factor kappa-B/Rel (NF-κB/Rel) comprises a group of transcription factors encoded by a family of evolutionarily conserved genes [5]. In cells, these factors can be potently activated by the pleiotropic cytokine, tumor necrosis factor (TNF)-α, a molecule that plays a

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key role in development, immunity, and inflammation [6]. The activation of NF- κ B by this cytokine triggers a transcriptional induction of a wide array of genes central for coordinating the immune, inflammatory, and tissue repair responses to injury, microbial infection, and stress [7]. These induced genes, which are pivotal to mediating these defenses, include adhesion molecules, cytokines, chemokines, growth factors, and inducible enzymes [7].

Remarkably, in recent years, an important additional function has been ascribed to NF- κ B -- that is, the promotion of cellular survival through a blockade of PCD [8]. Through this activity, NF- κ B participates in diverse biological processes, which serve beyond the conceptual boundaries of the immune system to include embryogenesis, and homeostasis and function of liver, skin, and central nervous system [8]. In fact, the anti-apoptotic action of NF- κ B has received increasing recognition for playing a central role in the pathogenesis of cancer as well as chronic inflammatory diseases such as rheumatoid arthritis (RA) and inflammatory bowel disease (IBD) [8,9,10]. Consequently, many standard therapeutic treatments to these conditions attempt to inhibit this anti-apoptotic activity of NF- κ B [9,10,11]. However, such therapies often have, as a major obstacle, serious unintended side effects. Most notably, global blockers of NF- κ B, such as corticosteroids, and non-steroidal anti-inflammatory drug (NSAIDs) such as aspirins, can cause severe immunosuppressive effects, which have limited their clinical use.

In a ray of hope, an evolving view of NF- κ B is that its actions in immunity and in promoting cell survival are executed through independent subsets of target genes. Recent studies of NF- κ B and its ability to inhibit apoptosis support this notion. Several laboratories including our own, have elucidated that NF- κ B downregulates apoptosis through a crosstalk with the c-Jun-N-terminal (JNK) mitogen activated protein kinase (MAPK) pathway, during the triggering of a so-called death receptor (DR)-induced pathway [12–14]. Hence, this seems to suggest the possibility that novel, therapeutic drugs may one day be obtained to selectively target the anti-apoptotic actions of NF- κ B, at the level of its specific effectors, without significantly affecting its capacity to normally regulate the coordination of immune and inflammatory processes.

In this article, we discuss how NF- κ B, through the regulation of its anti-apoptotic effectors, leads to suppression of this JNK cascade. In fact, a handful of NF- κ B-inducible factors seem to mediate this crosstalk through distinct and separate mechanisms. One of these mechanisms involves a restraint of ROS accumulation following apoptotic signaling through TNF α -receptors (TNF-Rs) [15,16,17]. Herein, findings from recent studies are presented and their relevance to physiology and inflammation is discussed.

INHIBITION OF APOPTOSIS BY NF- κ B

NF- κ B transcription factors are complexes comprised of homo- or heterodimeric combinations of five members of the NF- κ B family of polypeptides [5,7,8]. In mammalian cells, they consist of Rel (c-Rel), RelA (p65), RelB, p50/p105 (NF- κ B1), and p52/p100 (NF- κ B2) [5,7,8]. The proteins are expressed in virtually all tissues, and the most abundantly found NF- κ B complex is the RelA(p65)-p50 heterodimer [5,7,8]. In unstimulated cells, inactive NF- κ B dimers are found in the cytoplasm, sequestered to inhibitory I κ B poly-peptides, and can be rapidly activated by an array of stimuli that triggers the phosphorylation and proteasome-dependent proteolysis of the I κ B proteins [5,7,8]. This phosphorylation of I κ B proteins depends upon an upstream protein kinase complex, known as the IKK complex [5,7,8]. Liberated NF- κ B complexes, free of the I κ B proteins, then rapidly enter the nucleus and induce transcription of coordinate sets of target genes which regulate inflammation, immunity, and inhibition of apoptosis [5,7,8].

Several groups originally revealed the anti-apoptotic function of NF- κ B, in studies involving the use of genetically modified mice [18,19] or specific NF- κ B inhibitory molecules [20–22]. It was later found that deletions of genes encoding members of the NF- κ B family result in

profound defects in development and tissue homeostasis [reviewed in 8], in part caused by excessive apoptosis induced through TNF-Rs [23,24]. For example, *relA*-deficient mice succumb to embryonic lethality caused by massive liver apoptosis [18]. Mouse embryonic fibroblasts (MEFs) derived from these animals also show increased sensitivity to apoptosis [19]. Moreover, combined gene inactivation of other NF- κ B family members that generate compound null mutations revealed redundant anti-apoptotic roles between subunits of this transcription factor [8].

When dysregulated, the anti-apoptotic action of NF- κ B can cause human diseases. Indeed, it has received increasing recognition for playing a central role in oncogenesis and chemoresistance of certain cancers [8–11]. Constitutive high levels of NF- κ B activity are required to maintain survival of a number of human tumors, such as Hodgkin's Lymphoma, diffuse large B-cell lymphoma (DLBCL), multiple myeloma (MM), chronic myelogenous leukemia (CML), and breast cancer [8,9]. The anti-apoptotic activity of NF- κ B has also been found to be essential for transformation caused by oncogenes such as oncogenic forms of Ras (e.g H-Ras[G12V] and K-Ras[G12D] mutants) and Bcr-Abl, and by those encoded in certain viral genomes, such as the Epstein Bar Virus (EBV) genome [8,9].

For the treatment of cancer in humans, it is now being appreciated that approaches that attempt to inhibit the pro-survival effects of NF- κ B, aid in the therapeutic efficiency of such treatments [9,11]. In certain cancer cells, experimental inhibition of the transcription factors seems to improve the therapeutic apoptotic response to ionizing radiation as well as to daunorubicin chemotherapy [9,11]. Furthermore, glucocorticoids, which block NF- κ B, are being included as part of the therapeutic regimen for Hodgkin's lymphoma. More recently, proteasome inhibitor (e.g. PS-341) has been used with success for the treatment of patients with MM, and in fact, these drugs appear to also have beneficial effects against cancers of the prostate and lung, as well as lymphoma [8,9,11]. Of note, selective inhibitors of the IKK β component of the IKK complex represent another class of promising new drugs in anti-cancer therapy [11].

THE NF- κ B-MEDIATED CONTROL OF APOPTOSIS INDUCED BY TNF-RS

The antagonism of apoptosis by NF- κ B has been best recognized and most well-understood in the context of PCD induced by the so-called death receptors (DRs), such as TNF-R1 [6,8]. This receptor is usually activated by engagement with its natural ligand, TNF α , a pleiotropic cytokine capable of exerting diverse physiological influence on inflammation, immunity, hematopoiesis, and morphogenesis [6]. Although TNF α possesses an additional receptor, TNF-R2, its apoptotic effects have been best characterized through its engagement with TNF-R1 [6]. Curiously, however, although signal transduction initiated through TNF-R1 is highly capable of eliciting PCD [6], most cells are resistant to cell death activated by this receptor. This is because following stimulation of TNF-R1, NF- κ B becomes potently activated [6,8].

Indeed, this functional duality between PCD and cell survival induced by TNF α stems from competing signal transduction pathways that are concomitantly activated in cells [6,8,25,26]. The apoptotic response stimulated by TNF-R1 depends upon the sequential recruitment and activity of several proteins [6,25,26]. Ligand engagement of this receptor rapidly activates the formation of complex I, composed of TNF-R1-associated death domain (TRADD), TNF receptor-associated factor (TRAF)-2, as well as receptor interacting protein-1 (RIP1) [6,25, 26]. These events then lead to activation of NF- κ B and other pathways, ultimately resulting in cell survival [6,25,26]. Multiple reports now indicate that following its formation at the inner side of the plasma membrane, if modified, complex I can subsequently localize to the cytoplasm, where a second phase of protein recruitment occurs, thereby creating complex II [25,26]. Herein, Fas-associated death domain (FADD) and caspase-8 and -10 proteins associate sequentially, ultimately leading to apoptosis [25,26].

Activation of NF- κ B by TNF α effectively blocks the PCD triggered by the cytokine, and it is now clear that NF- κ B activation is obligatory to antagonize TNF α -triggered killing [6,8]. MEFs derived from *relA*-deficient mouse embryos show high sensitivity to TNF α -induced apoptosis [19], as do other cells that have other forms of NF- κ B deficiencies [12,13,20–22]. In large part, the inhibition of TNF α -triggered PCD by NF- κ B is mediated through transcriptional induction of cytoprotective target genes [6,8]. In general, this explains the results observed over the years, that unless inhibitors of RNA or protein synthesis are provided, most cells resist the induction of PCD by TNF α [6,8].

The induced cytoprotective effectors of NF- κ B have been of high interest and some have been characterized in recent years [6,8]. Each has been demonstrated to block the execution of TNF α -induced apoptosis through a distinct set of mechanisms [6,8]. For example, cellular FLICE-inhibitory protein (c-FLIP) targets early signal transduction events proximal to the TNF-R1, and likely prohibits caspase-8 activation [6,8]. A1/Bfl-1, Bcl-x_L, and Bcl-2 are members of the well-known anti-apoptotic Bcl-2 family of proteins. Blockade of apoptosis by these factors appears to involve inhibition of mitochondrial membrane permeability [6,8]. Furthermore, IAP-1/2 and TRAF-1/2 may block apoptosis through a feedback regulation of receptor signaling [6,8]. More recently, it has been shown that Spi2A, another target of NF- κ B, inhibits cathepsin B to block the lysosomal pathway of PCD [27].

PRO-APOPTOTIC ROLE OF THE JNK MAPK PATHWAY

The JNK pathway, also known as the stress activated protein kinase (SAPK) pathway, represents one of at least three major mitogen activated protein kinase (MAPK) cascades [28,29]. The others include the p38- and the extracellular-regulated kinases (ERK) MAPKs [28,29]. Functionally, all three cascades act to transduce, amplify, and integrate signals from a diverse array of cellular stimuli in order to elicit appropriate physiological responses [28, 29]. This is achieved through a signal transduction that involves sequential phosphorylation events mediated by numerous kinases, in a precisely regulated and highly complex process that is far from being fully understood [28,29]. The three members that comprise the JNKs are each encoded by distinct genes, JNK1/2/3 [28]. In cells, the activation of the JNK group of MAPKs has primarily been associated with proinflammatory cytokines such as IL-1 β and TNF α , as well as various forms of stress stimuli such as UV radiation, pH, or hypoxic stress [28]. In response to these stimuli, JNKs have been known to induce proliferation, differentiation, or apoptosis [28]. By these actions, JNK seems capable of contributing to morphogenesis during development and to tumorigenesis [28]. Further, a dysregulation of JNK activities has been associated with human disease [28,30].

Prior to the discovery of a crosstalk between NF- κ B and JNK, an involvement of JNKs with apoptosis had been clearly established by studies of JNK null mutations in mice. MEFs from (*Jnk1*^{-/-}, *Jnk2*^{-/-}) mice were found to be almost completely resistant to PCD induced by genotoxic stress stimuli such as UV radiation, exhibiting reduced cytochrome c release and activation of caspase-3 in response to these stimuli [31]. Moreover, *Jnk1*^{-/-} or *Jnk2*^{-/-} thymocytes are refractory to death induced by the triggering of CD3, a protein component of the T-cell antigen receptor, *in vivo* [32]. Also, neurons from *Jnk3*^{-/-} mice are severely defective in their apoptotic response to excitotoxins [28].

TAKING AIM ON THE JNK SIGNALING PATHWAY: NF- κ B-INDUCIBLE EFFECTORS TARGETING JNK ACTIVATION

It is becoming increasingly recognized that to ensure thorough inhibition of apoptosis triggered by TNF-Rs, NF- κ B targets the JNK cascade as an important means to secure cellular survival [12–14]. Under normal circumstances, TNF α triggers only a transient activation of the JNK

cascade. This activity of the cascade is rapidly increased within minutes of ligand engagement of TNF-Rs, only to return to basal levels usually within one hour [12–14]. However, an inhibition of NF- κ B by either ablation of RelA or IKK β (a catalytic subunit of the IKK complex) or by expression of I κ B α M (a degradation resistant I κ B protein) has revealed a second phase of JNK activation [12–14]. Indeed, it is this later induction of JNK signaling that has been linked to apoptosis [12–14]. In support of this view, it has been shown that expression of MKK7-JNK fusion proteins, which mimic constitutively active JNK, is sufficient to induce apoptosis [33]. Consequently, many investigators have come to view NF- κ B as a factor required for restraining this persistent phase of JNK activation following TNF α stimulation, and furthermore, have appreciated this activity as being crucial for inhibition of cell death downstream of TNF-Rs [12–14]. In fact, a suppression of JNK signaling by pharmacological agents or expression of dominant negative kinase mutants effectively rescues NF- κ B null cells from TNF α -mediated cell death [12–14]. Likewise, in the absence of NF- κ B, a knockdown of MKK7 expression or a genetic inactivation of *Jnk1* and *Jnk2* abrogates cell death triggered by TNF α [35,36]. Interestingly, recent studies indicate that JNK inhibition may also block the necrosis-like response induced by TNF α in NF- κ B null cells [17] (discussed below).

Indeed, studies in animal models have bolstered the relevance of the antagonistic crosstalk between NF- κ B and JNK for cell survival. An inhibition of NF- κ B activation in mouse livers, through a conditional deletion of IKK β , results in increased activation of JNK during a systemic challenge with concanavalin A (ConA) [37], a treatment that provokes tissue damage through TNF-Rs. In fact, it seems that this induction of JNK correlates with liver injury [37]. Accordingly, targeted deletion of either *Jnk1* or *Jnk2*, in the IKK β -inactivated mice, dramatically diminishes the tissue injury by the systemic challenge with ConA [37]. Thus, NF- κ B acts to suppress the JNK pathway in order to reduce liver injury *in vivo*.

A recent study by Deng and colleagues has introduced a possible mechanism that may help to clarify the cytotoxic role of JNK in TNF α -induced cell death [35]. The model suggests that activation of JNK by TNF-Rs causes death by promoting the formation of jBid, a novel cleaved form of Bid, generated through a caspase-8-independent mechanism [35] (see Fig. 1). jBid then seems to target mitochondria and trigger the selective release of the apoptogenic factor Smac/Diablo, but not of cytochrome c, into the cytosol [35]. Here, Smac/Diablo associates with and inhibits inhibitor of apoptosis proteins (IAPs), leading to activation of caspase-8 and ultimately, of PCD [35] (Fig. 1). Consistent with this view, a downregulation of Smac/Diablo by RNA-interference diminishes TNF α -induced activation of caspase-8 and PCD [35]. Nevertheless, exactly how JNK influences the formation of jBid is not known. Further, it is possible that there are other mechanisms by which JNK promotes cytotoxicity triggered by TNF α .

Importantly, it seems that the effect of NF- κ B on the JNK cascade is not a direct consequence of caspase inhibition. This is because in NF- κ B-deficient cells, even after treatment with the caspase blocker z-VAD_{fmk}, JNK activity induced by TNF α remains sustained [13,14]. This is important since caspases are known to have the ability to activate MAP3Ks [28]. Hence, collectively, abundant evidence in the literature now suggests that NF- κ B intersects with the JNK pathway and that a common outcome of this intersection is the control of apoptosis triggered by TNF α .

The notion that the JNK pathway plays a pro-apoptotic role is not without exception, however, as JNK may not promote apoptosis in all circumstances. In fact, the role of JNK is complex, having been shown in different systems to promote apoptosis, necrosis, and even survival [12–17,38]. A recent study using fibroblasts double deficient in *Jnk1* and *Jnk2* (*Jnk1*^{-/-}*Jnk2*^{-/-}) has shown that these cells are protected against apoptosis in response to TNF α , suggesting that in the presence of functional NF- κ B complexes, the JNK pathway plays

a role in facilitating cell survival [38]. Yet, in these same cells, JNK is also required to promote a necrosis-like response downstream of TNF-Rs [17], and in fact, it appears that the overall result of JNK knockout deletions is a net increase in cell survival [17]. Most likely, the outcome of JNK signaling depends upon stimulus- and tissue-specific factors [28], involving integration and/or crosstalk of the JNK pathway with other signaling pathways, such as ERK and Akt/PKB, activated at the same time by TNF α [36,38]. Indeed, it may be that survival signaling by JNK involves transient JNK activation, affecting gene expression [38], whereas its roles on cell death by TNF α relies upon the sustained phase of JNK activity [12–17,32,34,36].

NF- κ B-INDUCIBLE EFFECTORS TARGETING THE JNK PATHWAY

The primary mechanism mediated by NF- κ B to secure an effective shutdown of the JNK pathway following triggering of TNF-Rs, and therefore, inhibition of apoptosis, appears to involve the activation of transcriptional target genes [6,8,35] (see Fig. 1). Several such targets capable of blocking the JNK pathway have been elucidated and are described immediately below.

A20

In response to TNF α , NF- κ B rapidly induces transcription of zinc-finger protein A20 [8,39]. This factor seems to be a critical mediator of NF- κ B for downregulation of the JNK pathway. A20-deficient MEFs display sustained activation of JNK and increased apoptosis in response to triggering of TNF-Rs [39,40]. Notably, however, overexpression of A20 fails to block PCD in NF- κ B-deficient cells [8]. Thus, although A20 seems to be essential for the prosurvival activity of NF- κ B, this factor alone fails to completely explain the action of NF- κ B on JNK signaling and PCD downstream of TNF-Rs. Furthermore, the mechanism by which A20 diminishes JNK signaling remains elusive. On the one hand, A20 represents a key negative feedback mechanism responsible for inhibition of NF- κ B during TNF α -triggered signal transduction [39,40]. It was recently shown that this downregulation of the NF- κ B pathway by A20 involves A20-mediated ubiquitination and inactivation of RIP1 [41], a factor required for activation of NF- κ B by TNF-Rs [6]. On the other hand, because RIP1 has no role in activation of JNK downstream of TNF-Rs [6], the A20-mediated inhibition of the JNK pathway is likely mediated through some other mechanism. For instance, A20 is capable of associating with TRAF2 [6,39], which is required for activation of JNK by TNF α [28], and is recruited to the TNF-R1 complex upon receptor engagement with TNF α [42]. Thus, perhaps the A20-mediated blockade of JNK signaling involves inactivation of TRAF2 or of another molecule proximal to TNF-R1 [6,39,40]. Consistent with this notion, A20 selectively hinders signaling through TNF-Rs, while signaling through IL-1 β receptor is normal in A20^{-/-} MEFs [39,40]. Moreover, additional biochemical studies have suggested that A20 may have a broader role in the regulation of MAPKKK activity and TNF-R signaling [6,40]. Thus, the exact mechanism for how A20 mediates downregulation of the JNK cascade has yet to be described.

Gadd45 β

We have previously shown that *Gadd45 β* , an immediate-early gene [43], is another key component of the mechanism by which NF- κ B downmodulates the JNK cascade [12]. *Gadd45 β* belongs to a family of related genes, which also include *Gadd45 α* and *Gadd45 γ* [43]. Interestingly, these factors have been implicated in multiple processes, including cell cycle control, DNA repair, and regulation of MAPKs [12,34,43,44]. In response to TNF α , NF- κ B upregulates expression of *Gadd45 β* [12]. Furthermore, ectopic expression of *Gadd45 β* in NF- κ B null cells suppresses TNF α -induced activation of JNK [12]. Importantly, inactivation of *Gadd45 β* can disrupt the suppression of JNK signaling induced by TNF α [12,34], which indicates that *Gadd45 β* is necessary for the control of this signaling by NF- κ B.

A recent study by our group has shown that the Gadd45 β -mediated suppression of JNK signaling involves inhibition of the upstream kinase of JNK, MKK7/JNKK2 [34]. This study demonstrates a direct binding of Gadd45 β to MKK7, which blocks the catalytic activity of the kinase, most likely by preventing its access to ATP [34]. Consistent with the view that Gadd45 β serves as an effector downstream of NF- κ B, NF- κ B-deficient cells fail to downregulate MKK7 activity induced by TNF α [34]. Thus, the interaction between Gadd45 β and MKK7 represents a critical molecular link between the NF- κ B and JNK pathways.

Work in another laboratory has suggested that knockout mutation of Gadd45 β in MEFs causes no apparent defect on PCD induced by TNF α [45]. Yet, it seems that a functional redundancy for Gadd45 β function may account for this apparent discrepancy between published reports [34,46]. Namely, a blockade of MKK7 activation by TNF α in MEFs appears to be controlled also by factor(s) other than Gadd45 β [34]. Nevertheless, despite this redundancy in the inhibition of MKK7 induced by TNF-Rs, acute inhibition of Gadd45 β by cell-permeable peptides has revealed an essential role for Gadd45 β in blocking TNF α -induced PCD [34].

Indeed, since MKK7 represents the dominant and specific kinase activator of JNK induced by TNF α [28,47], the blockade of MKK7 seems to suitably explain the effects of Gadd45 β on the JNK pathway [12,34]. In contrast, the associations of Gadd45 β with other MAPKKs, such as MEKK4/MTK1 and ASK1/MEKK5, do not appear relevant to the ability of Gadd45 β to control the activation of JNK downstream of TNF-Rs. This is because MEKK4 is not involved in TNF α -induced signaling [12,28], and activity of ASK1 appears to be unaffected by an association with Gadd45 β [34].

XIAP

In addition to an upregulation of Gadd45 β , another mechanism by which NF- κ B controls the JNK pathway has been suggested to involve an induction of XIAP [8]. This factor is a member of the IAP family of inhibitors of caspases and is capable of binding to and inactivating caspase-3 and -7 [48]. XIAP is a known target of NF- κ B, and its overexpression in NF- κ B-deficient cells reduces TNF α -induced cytotoxicity [49]. In fact, thymocytes of XIAP transgenic mice are resistant to PCD induced by diverse stimuli [48]. Furthermore, an overexpression of XIAP in *relA*^{-/-} cells reduces activation of JNK downstream of TNF-Rs, while having no effect on activation of p38 and ERK [13]. Notably, XIAP is able to inhibit both the caspase-dependent and the caspase-independent phases of JNK induction by TNF α [13]. Thus, its action on MAPK signaling is unlikely due to its inhibitory activity on caspases alone [48]. Nonetheless, how XIAP mediates downmodulation of the JNK cascade remains unclear. While XIAP is able to interact with kinases within the JNK pathway [48,50], this interaction of XIAP seems to promote an activation of JNK, rather than its inhibition. Finally, *xiap*-deficient mice show no obvious apoptotic phenotype [8], and in fact, ablation of XIAP in MEFs causes no defect in JNK activation in response to triggering of TNF-Rs [9]. Clearly, further studies are needed to determine the physiological relevance of the XIAP-mediated regulation of the JNK pathway.

INDIRECT TARGETING OF THE JNK PATHWAY BY NF- κ B THROUGH THE CONTROL OF REACTIVE OXYGEN SPECIES (ROS)

Recent reports have pointed to an NF- κ B-dependent role in the control of the accumulation of reactive oxygen species (ROS) in response to TNF α . ROS play a critical role in PCD triggered by various stimuli, including radiation and chemotherapeutic agents [51]. Importantly, they have been shown to be key mediators of cell death induced by TNF-Rs [6,15–17]. In comparison to their wild-type counterparts, NF- κ B-deficient cells aberrantly accumulate ROS in response to TNF α stimulation [15–17]. Various anti-oxidant agents that neutralize ROS are,

in fact, capable of protecting these NF- κ B null cells from TNF-R-induced cell death [15,16]. Thus, an inhibition of ROS accumulation seems to represent a key mechanism by which NF- κ B blocks TNF α -induced cytotoxicity [15–17].

Cytotoxicity by ROS is mediated in part by the JNK pathway [15,16,52]. In particular, it seems that ROS are required for sustained activation of JNK by TNF-Rs [15–17,52]. This sustained activity of JNK, downstream of TNF-Rs, depends in part on the TRAF2-binding MAPKKK, ASK1/MEKK5 [52]. In turn, the activity of ASK1/MEKK1 relies upon ROS, since regulation of the ASK1 inhibitor, thioredoxin (Trx), involves a redox-dependent mechanism [52,53]. Thus, in the TNF-R-induced signaling pathway, ROS lie upstream of JNK.

Interestingly, new data in a recent study have shown that ROS induction in this pathway also occurs downstream of activation of JNK [17]. In this study, it was shown that *Jnk1^{-/-}Jnk2^{-/-}* fibroblasts lacking functional NF- κ B display an inability to produce ROS during TNF α stimulation. In contrast, their *Jnk1^{+/+}Jnk2^{+/+}* counterpart (which also were lacking NF- κ B) exhibited dramatic accumulation of ROS following the triggering of TNF-Rs [17]. From this, it was suggested that a reciprocal relationship between JNK and ROS exist, whereby JNK activity might be critical for ROS production [17]. Indeed, JNK and ROS may participate in a positive feedback mechanism that amplifies and ultimately induces cell death. It should be cautioned, though, that ROS production also occurs in mitochondria upon uncoupling of the oxidative respiratory chain. Thus, it is possible that the induction of ROS observed in *Jnk1^{+/+}Jnk2^{+/+}* (but not in *Jnk1^{-/-}Jnk2^{-/-}*), NF- κ B-deficient cells is partly a consequence, rather than a cause of cell death. A dynamic relationship between ROS and JNK, however, is likely to exist.

The interplay between ROS and JNK activated by TNF-Rs also seems to determine how a cell dies. Notably, JNK-deficient cells were reported to die predominantly through a necrotic-like, cell death mechanism [17]. Thus, it seems that, depending on the cell system, TNF α is capable of eliciting cell death by both apoptosis and necrosis-like (i.e. caspase-independent) mechanisms [15–17]. Experimental conditions may, indeed, determine the exact form of cell death that predominates during activation of TNF-Rs. Regardless of the precise nature of the mechanism by which cells succumb to TNF α -induced cytotoxicity, however, activation of NF- κ B appears to be capable of blocking both types of death response [15–17]. Furthermore, irrespective of the relative positioning of ROS with respect to JNK, ROS is still recognized to be a major cell death-inducing element triggered by TNF-Rs [15–17]. Indeed, we and others have revealed an essential role of NF- κ B to the control of ROS generation triggered by TNF-Rs [15–17]. An upregulation of at least two target genes of NF- κ B has been proposed to account for this NF- κ B-dependent restraint of redox disequilibrium [6,8,16].

Ferritin Heavy Chain

Using a gene array-based screen, we have recently identified Ferritin heavy chain (FHC) as a critical mediator of the antioxidant and anti-apoptotic actions of NF- κ B [16]. FHC represents one of two subunits of Ferritin, the major iron storage mechanism in cells [54]. FHC is upregulated by TNF α through a mechanism controlled by NF- κ B, is essential for inhibition of TNF α -induced PCD, and blocks apoptosis in NF- κ B-deficient cells [16]. This blockade of apoptosis by FHC involves a suppression of ROS accumulation in response to TNF α stimulation, and this restraint prevents sustained activation of the JNK pathway [16]. The antioxidant action of FHC depends on sequestration of iron [16]. This is crucial, since iron is a transition metal capable of catalyzing the generation of ROS through the Fenton reaction, leading to formation of highly reactive hydroxyl radicals ($\cdot$ OH) [54]. Notably, knockdown experiments suggest that in some tissues, FHC may represent a significant proportion of protective function activated by NF- κ B for inhibition of TNF-R-induced JNK signaling and apoptosis [16].

Physiologically, an upregulation of FHC occurs in the liver during acute-phase responses to stress, injury, and infection [54]. Proinflammatory cytokines trigger this upregulation and it is this increase in FHC synthesis that seems to contribute to hypoferrremia during chronic inflammation [54]. Interestingly, through this mechanism, NF- κ B may restrict systemic iron availability to exert its protective effects at distant sites, such as at sites of inflammation where elevated levels of TNF α could potentially cause extensive ROS-mediated, tissue injury [10, 55]. FHC also seems to play an important role in cancer [54]. In certain late-stage cancers, the protective action of FHC antagonizes oxidative and genotoxic stress [54]. Moreover, high levels of FHC in tumors have been associated with their resistance to anticancer treatment and an aggressive phenotype [54]. Thus, FHC may contribute to NF- κ B-dependent chemoresistance in cancer, and therefore, represent an appropriate target for anti-inflammatory and anti-cancer therapy.

Mn-SOD

Mn⁺⁺ superoxide dismutase (Mn-SOD), a mitochondrial enzyme that catalyzes dismutation of superoxide anion ($O_2^{\cdot-}$) into hydrogen peroxide (H_2O_2), has also been suggested to play a role in the antioxidant and protective actions of NF- κ B [6,8,56,57]. This is because Mn-SOD is upregulated by TNF α , under the transcriptional control of NF- κ B [6,8,56,57]. Furthermore, in some systems, Mn-SOD can inhibit TNF α -induced cytotoxicity [16,56,57]. However, the relative significance of Mn-SOD is still a matter of debate. This is so, since in NF- κ B-deficient cells, ectopic expression of Mn-SOD confers modest or no protection against TNF α -induced cell death [15,16]. Moreover, Mn-SOD appears to be relevant to the anti-apoptotic action of NF- κ B only in certain tissues, since in others, neither its basal nor TNF α -activated level is modulated by NF- κ B [16,57,58]. Nevertheless, despite that it is insufficient at blocking PCD triggered by TNF-Rs [16,17], Mn-SOD may still be necessary for controlling TNF α -induced changes of cellular redox levels. Indeed, for an effective control of ROS, synergistic actions of FHC and Mn-SOD may be key. This may explain the failure of Mn-SOD expression at blocking TNF α -induced apoptosis in NF- κ B null cells [15,16]. It is conceivable that while upregulation of Mn-SOD promotes dismutation of $O_2^{\cdot-}$ into H_2O_2 , sequestration of iron by FHC permits clearance of H_2O_2 by catalases and peroxidases. However, in NF- κ B-deficient cells or in other biological situations where FHC levels are low, iron may still remain available enough to catalyze the reduction of H_2O_2 to produce highly reactive OH radicals, and signal PCD [15,16].

The coordinate inductions of antioxidant genes, FHC and Mn-SOD by NF- κ B, seem to provide a new link between the NF- κ B and JNK pathways, downstream of TNF-Rs. Namely, NF- κ B appears to halt the JNK cascade by at least two distinct mechanisms: directly, through upregulation of Gadd45 β , A20 and XIAP; and indirectly, through an upregulation of FHC and of Mn-SOD, which together restrain accumulation of ROS. It is probable that to ensure effective suppression of JNK signaling, these factors act cooperatively (see Fig. 1). Nevertheless, the inhibition of the JNK pathway that is activated by NF- κ B remains subject to modulation by tissue- and stimulus-specific factors. Indeed, within diverse biological contexts, this signal transduction network may remain adaptable, and allow an organism to orchestrate suitable responses to apoptotic stimuli.

POTENTIAL NEW AVENUES FOR THERAPY OF CHRONIC INFLAMMATORY DISEASES AND CANCER

The NF- κ B-mediated control of TNF-R-induced PCD has consequences to human diseases [8–10]. A positive feedback mechanism exists between TNF α and NF- κ B-TNF α is a strong activator of NF- κ B, which in turn, is a strong transcriptional activator of TNF α [6,8,10]. This relationship is pivotal to the pathogenesis of chronic inflammatory conditions such as

rheumatoid arthritis (RA) and inflammatory bowel disease (IBD) [10,55]. In fact, some treatments for these conditions include drugs that either aim to inhibit NF- κ B, such as aspirin and glucocorticoids, or seek to neutralize the actions of TNF α , such as anti-TNF α neutralizing antibodies [10,11,55]. Likewise, glucocorticoids and proteasome inhibitors, which also block NF- κ B, have been used with some success to treat human malignancies such as HL and MM [8,9,11]. Unfortunately, these drugs can achieve only partial inhibition of NF- κ B and furthermore, they have serious side effects that severely hamper their clinical use. Thus, more effective therapeutic approaches should attempt to develop strategies that target the downstream anti-apoptotic effectors of NF- κ B, rather than NF- κ B itself.

A new opportunity seems to have now presented itself with the discovery that NF- κ B directs a suppression of the JNK cascade. Perhaps by interfering with the NF- κ B-mediated inhibition of JNK signaling, a progression of apoptosis can occur in self-reactive and proinflammatory cells within sites of inflammation, where high concentrations of TNF α are found [10,55]. In this view, agents that hinder iron sequestration by FHC or that disrupt association between Gadd45 β and MKK7 may indeed accomplish this objective [16,34]. Presumably, novel drugs that might interrupt the crosstalk between NF- κ B and JNK may mediate an uncoupling of the anti-apoptotic and proinflammatory actions of NF- κ B, and therefore, side step the harmful side effects of general NF- κ B inhibitors [8,9,11,55]. Moreover, since the inhibition of JNK by NF- κ B appears to manifest a degree of cell-type specificity [34,45,46], such drugs might also achieve selective targeting of this inhibition in diseased tissues.

The relevance of this new therapeutic approach may also apply to cancer. In most tumors, JNK and NF- κ B seem to have opposing effects. While activators of the JNK pathways (e.g. MKK4, JNK3, and BRCA1) act as tumor suppressors [30], NF- κ B has been shown to block transformation-induced apoptosis [8,9]. Indeed, cancer cells seem to require constitutive activation of NF- κ B to suppress JNK-mediated PCD induced by oncogene products, such as oncogenic Ras and Her-2/Neu, which are often potent inducers of JNK [28,30]. Of note, apoptosis caused by certain anti-cancer agents such as topoisomerase inhibitors, requires JNK [28], but is antagonized by NF- κ B [8,9]. Hence, an augmentation of JNK signaling, achieved through inhibition of selected NF- κ B targets, may represent a powerful new line of attack for anti-cancer treatment.

CONCLUDING REMARKS

The restraint on JNK signaling controlled by NF- κ B is critical for several physiological processes, as well as for chronic inflammation and cancer. In recent years, the identification of NF- κ B-inducible inhibitors of the JNK cascade has advanced our understanding of how this crosstalk is mediated. The redundancy reflected in having multiple effectors may be critical for ensuring an effective shutdown of the JNK cascade and furthermore, might allow an organism to modulate the anti-apoptotic response according to specific biological contexts and needs. Undoubtedly, it would be of biological interest to determine the role(s) of the individual NF- κ B-inducible gene, most crucial in each of these contexts and to evaluate how their products inhibit the JNK cascade. Establishment of conditional knockout models will be vital for addressing these key issues. Furthermore, because inappropriate blockade of apoptosis by NF- κ B seems to underlie certain human diseases, these endeavors will likely facilitate the development of novel, more selective approaches for treatment of these conditions.

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Fig. 1. Pathways mediating inhibition of TNF-R1-induced JNK signaling and apoptosis by NF-κB. Shown are formation of complex I, leading to NF-κB activation, induction of protective genes, JNK inhibition, and cell survival; and complex II, leading to caspase-8/10-mediated cleavage of Bid into tBid, which then targets mitochondria to induce cytochrome c release and, ultimately, cell death. The figure also depicts JNK activation, which results in formation of jBid, which promotes PCD by triggering Smac/Diablo release into cytosol, inhibition of the c-IAP1-TRAF2 complex and consequent activation of caspase-8.