Histol Histopathol (2006) 21: 69-80

http://www.hh.um.es

Histology and Histopathology

Cellular and Molecular Biology

Review

The NF-κB-mediated control of ROS and JNK signaling

C. Bubici, S. Papa, C.G. Pham, F. Zazzeroni* and G. Franzoso

The Ben May Institute for Cancer Research, The University of Chicago, Chicago, USA

Summary. NF-κB/Rel transcription factors are best known for their roles in innate and adaptive immunity and inflammation. They also play a central role in promoting cell survival. This latter activity of NF-κB antagonizes programmed cell death (PCD) induced by the proinflammatory cytokine tumor necrosis factor (TNF)α and plays an important role in immunity, lymphopoiesis, osteogenesis, tumorigenesis and radio- and chemo-resistance in cancer. With regard to TNFα, the NF-κB-mediated inhibition of PCD seems to involve an attenuation of the c-Jun-N-terminal kinase (JNK) cascade mediated through the induction of select downstream targets such as the caspase inhibitor XIAP, the zinc-finger protein A20, and the inhibitor of the MKK7/JNKK2 kinase, Gadd45ß/Myd118. Notably, NF-κB also blunts accumulation of reactive oxygen species (ROS), which themselves are pivotal elements for induction of PCD by TNFα, and this suppression of ROS formation mediates an additional protective activity recently ascribed to NF-κB. The antioxidant activity of NF-κB has been shown to depend upon upregulation of both Ferritin heavy chain (FHC) – a component of Ferritin, the primary iron-storage protein complex found in cells – and of the mitochondrial enzyme Mn^{++} superoxide dismutase (Mn-SOD). Indeed, the inductions of Mn-SOD and FHC represent another important means through which NF-κB controls proapoptotic JNK signaling triggered by TNFα. These findings might enable the development of new, more targeted approaches to treatment of diseases sustained by a deregulated activity of NF-κB, including some cancers and chronic inflammatory conditions.

Key words: Apoptosis, Necrosis, Ferritin, Gadd45ß, Inflammation, Cancer, JNK, NF-κB, TNFα, and reactive oxygen species (ROS)

Offprint requests to: Dr, Guido Franzoso, The Ben May Institute for Cancer Research, The University of Chicago, 924 East 75th Street, Chicago, IL 60637, USA. e-mail: gfranzos@midway.uchicago.edu

*Current address: Department of Experimental Medicine, The University of L'Aquila, L'Aquila, Italy

Introduction

Programmed cell death (PCD) is a form of cellular suicide that plays a central role in animal physiology, being required for tissue homeostasis, organ development and elimination of potentially harmful cells such cancerous and virus-infected cells. In metazoa, virtually all cells are programmed to self-destruct (Danial and Korsmeyer, 2004), and the correct activation and execution of the death program is critical for the very existence of these organisms. Indeed, alterations of the PCD process are a major contributing factor in the onset of an array of human diseases, ranging from cancer to autoimmune and neurodegenerative disorders such Alzheimer's and Parkinson diseases (Johnstone et al., 2002; Rathmell and Thompson, 2002). Remarkably, a critical element in the cell's decision on whether to live or die appears to be its ability to activate NF-κB-family transcription factors (Kucharczak et al., 2003).

These are widely regarded as evolutionarily conserved coordinators of innate and adaptive immune and inflammatory responses (Li and Verma, 2002; Chen and Greene, 2004; Hayden and Ghosh, 2004). During these responses, NF-κB orchestrates in fact activation of critical defense genes, including those encoding various adhesion molecules, cytokines, chemokines, growth factors and proinflammatory enzymes. In 1996, approximately 10 years after its original discovery, NF-κB was assigned yet another important biological function – namely the control of PCD (Beg and Baltimore, 1996; Liu et al., 1996; Van Antwerp et al., 1996; Wang et al., 1996). This additional function of NF-κB immediately attracted major biomedical interest – which in fact has not faded until this day. This is in part because through this ability to suppress PCD, NF-κB seems to participate in numerous important biological processes, including embryogenesis, B and T lymphopoiesis, and development and homeostasis of the skin, bone, liver and central nervous system (Karin and Lin, 2002; Orlowski and Baldwin, 2002; Gerondakis and Strasser, 2003; Kucharczak et al., 2003). An exaggerated promotion of cell survival by NF-κB is also a key contributing factor in tumorigenesis, cancer

chemoresistance and chronic inflammatory diseases such as rheumatoid arthritis (RA) and inflammatory bowel disease (IBD) (Makarov, 2000; Orlowski and Baldwin, 2002; Kucharczak et al., 2003). The importance of the NF-κB-mediated suppression of PCD in the pathogenesis of human illnesses is underscored by the fact that inhibitors of NF-κB have become drugs of choice for treatment of some of these illnesses (Makarov, 2000; Tak and Firestein, 2001; Orlowski and Baldwin, 2002; Amit and Ben-Neriah, 2003; Karin et al., 2004). In recent years, remarkable progress has been made towards the understanding of the mechanisms governing tumor necrosis factor α (TNFα)-induced PCD and NF-κB-mediated survival. Findings from several laboratories, including our own, have in fact shown that the protective action of NF-κB against TNFα-induced cytotoxicity involves a crosstalk with the c-Jun-N-terminal kinase (JNK) mitogen activated protein kinase (MAPK) pathway (De Smaele et al., 2001; Javelaud and Besancon, 2001; Tang et al, 2001). Interestingly, new studies indicate that this crosstalk between the NF-κB and JNK pathways is mediated in part through an inhibition of the accumulation of ROS triggered by stimulation of TNF-Rs (Sakon et al., 2003; Pham et al., 2004). The current feeling is that this new, deeper understanding of the molecular mechanisms controlling cell fate downstream of TNF-Rs represents an unprecedented opportunity for developing new drugs capable of selective blockade of the prosurvival activity of NF-κB in diseased tissues, without affecting other important functions of NF-κB in immunity and inflammation.

In this article, we will focus on recent discoveries that have unveiled the mechanisms by which TNFα–activated NF-κB complexes restrain accumulation of ROS and, more broadly, engage in a negative crosstalk with the JNK MAPK pathway – a signaling pathway known to promotes PCD (Davis 2000; Papa et al. 2004a). We then go on to discuss the relevance of this negative crosstalk to the suppression of cell death in animal physiology and disease. For other aspects of the regulation and function of the NF-κB and JNK pathways, we refer the reader instead to some of the many excellent and broader-in-scope reviews that have been recently published on these subjects (Karin and Ben-Neriah, 2000; Silvermann and Maniatis, 2001; Li and Verma, 2002; Ghosh and Karin, 2002; Kucharczak et al., 2003; Chen and Greene, 2004; Hayden and Ghosh, 2004).

Biological relevance of the NF-κB-mediated control of PCD

NF-κB transcription factors are a group of homo- and heterodimeric complexes composed of nearly all possible combinations of the members of the NF-κB family of polypeptides, which in mammalian cells consists of Rel (c-Rel), RelA (p65), RelB, p50/p105 (NF-κB1), and p52/p100 (NF-κB2) (Kucharczak et al., 2003; Chen and Greene, 2004; Hayden and Ghosh, 2004). Typically, the most abundant of these complexes is a heterodimer of the subunits RelA and p50. In unstimulated cells, ubiquitous NF-κB dimers usually lie latent in the cytosol, trapped there by binding to inhibitory IκB-family proteins (reviewed in Li and Verma, 2002; Ghosh and Karin, 2002; Kucharczak et al., 2003; Chen and Greene, 2004; Hayden and Ghosh, 2004). From these cytosolic pools, NF-κB complexes can be rapidly activated by an array of stimuli capable of triggering the sequential phosphorylation and proteasome-mediated degradation of IκB proteins. Newly-liberated NF-κB complexes, can then migrate into the nuclei and activate transcription of coordinate sets of target genes, thereby promoting inflammation, immunity, cell growth and cell survival.

The protective function of NF-κB was originally revealed in studies involving the use of genetically modified cells or highly active variants of the NF-κB inhibitor, IκBα, in the context of the stimulation of TNF-Rs (Beg and Baltimore, 1996; Liu et al., 1996; Van Antwerp et al., 1996; Wang et al., 1996). Subsequent studies then showed that knockout mutations of genes encoding NF-κB-family polypeptides cause profound defects in development and homeostasis of various organs including the skin, the central nervous system, the bone, the liver and the lymphoid tissues (reviewed in Kucharczak et al., 2003). It is now evident that many of these developmental and homeostatic defects are owed to an excessive PCD, and that this excessive PCD often depends upon the triggering of receptors of the TNF-R superfamily. It was shown, for instance, that RelA-/- mice succumb in uterus due to massive apoptosis in the liver (Beg et al., 1995) and that compound mutation of either TNFα or TNF-R1 completely rescues these mice from embryonic lethality (Doi et al., 1999; Rosenfeld et al., 2000; Alcamo et al., 2001). Consistently, mouse embryonic fibroblasts (MEFs) derived from RelA null animals exhibit a strikingly high sensitivity to TNFα-induced PCD (Beg and Baltimore, 1996), as do most other cells that have various forms of NF-κB deficiency (Kucharczak et al, 2003). The concomitant inactivation of genes encoding two or more members of the NF-κB group revealed further redundant functions of these transcription factors in cell survival (Kucharczak et al, 2003).

In addition to playing an important physiological role, when deregulated the prosurvival action of NF-κB is a major contributing cause of widespread human diseases. For instance, an inappropriate suppression of PCD caused by constitutively active NF-κB is increasingly being recognized for participating in chemo- and radio-resistance in cancer and in the pathogenesis of a growing list of human tumors, including diffuse large B-cell lymphoma (DLBCL), multiple myeloma (MM), Hodgkin's Lymphoma (HL), chronic myelogenous leukemia (CML) and breast cancer (Orlowski and Baldwin, 2002; Kucharczak et al., 2003). The prosurvival activity of NF-κB is also involved in

counteracting oncogene-induced PCD during malignant cellular transformation (Orlowski and Baldwin, 2002; Kucharczak et al., 2003).

The relevance of the NF-κB-mediated suppression of cell death in human malignancies can be appreciated especially if one considers that NF-κB-inhibiting compounds are now drugs of choice for treatment of some of these malignancies (Orlowski and Baldwin, 2002; Kucharczak et al., 2003; Karin et al., 2004). One class of such anti-cancer compounds is constituted by glucocorticoids, currently included in the therapeutic regimen for HL. Proteasome inhibitors (e.g. PS-341), a distinct class of NF-κB-targeting drugs, are instead being used for treatment of patients with MM and appear to have beneficial effects against cancers of the prostate and the lung, as well as against certain lymphomas (Orlowski and Baldwin, 2002; Kucharczak et al., 2003; Karin et al., 2004). Blockers of IKKß, a catalytic component of the IKK kinase complex responsible for signal-induced phosphorylation of IκBs, represent another group of promising new compounds for use in anti-cancer therapy (Karin et al., 2004). Finally, inhibitors of NF-κB have been shown to improve the therapeutic response to ionizing radiation and standard chemotherapeutic agents such as topoisomerase inhibitors (Orlowski and Baldwin, 2002; Karin et al., 2004).

Bases for the NF-κB-mediated suppression of TNF-R-induced PCD

The prosurvival action of NF-κB was first discovered and is best understood in the context of so-called "death receptors" (DRs), a prototypic example of which is TNF-R1 (Kucharczak et al, 2003; Wajant et al., 2003). This receptor is ligated by TNFα, a pleiotropic cytokine that plays a critical role in development, inflammation, immunity and morphogenesis (Wajant et al., 2003). TNFα also engages TNF-R2, a less well-characterized receptor that is closely related to TNF-R1 (Wajant et al., 2003). Paradoxically, although TNF-Rs are well known for their ability to elicit PCD, in most cells stimulation of these receptor by TNFα is insufficient to cause death (Kucharczak et al., 2003; Wajant et al., 2003). This is due to the activation of NF-κB complexes, which effectively antagonize TNF-R-induced cytotoxicity (Kucharczak et al., 2003).

The functional dichotomy of TNF-R1 seemingly stems from the ability of this receptor to activate opposing signal transduction pathways (Micheau and Tschopp, 2003; Wajant et al., 2003; Schneider-Brachert et al., 2004). Indeed, it was recently shown that the biological outcome to the ligation of TNF-R1 depends upon sequential assembly and activation of at least two multi-proteic complexes at the cytosolic tail of this receptor (Micheau and Tschopp, 2003; Schneider-Brachert et al., 2004): Complex I, consisting of the adaptors TNF-R1-associated death domain (TRADD) and TNF receptor-associated factor (TRAF)-2 along with the kinase receptor interacting protein-1 (RIP1), forms rapidly at the inner side of the plasma membrane and leads to activation of the c-Jun N-terminal kinase (JNK) cascade and NF-κB, which promotes cell survival (Micheau and Tschopp, 2003; Wajant et al., 2003; Schneider-Brachert et al., 2004). Complex II, also known as the "death-inducing signaling complex" (DISC), consists instead of TRADD, RIP1, Fas-associated death domain (FADD) and caspase-8 and appears to assemble on endocytic vescicles (so-called "receptosomes") in association with TNF-R1 (Micheau and Tschopp, 2003; Wajant et al., 2003; Schneider-Brachert et al., 2004). Here, caspase-8 becomes active through auto-catalytic cleavage, causing proteolytic processing of Bid (a "BH3-only" member of the Bcl-2 family) into tBid, which then targets mitochondria to induce mitochondrial outer membrane permeabilization (MOMP) and cytosolic release of cytochrome c and other factors, ultimately leading to PCD (Wajant et al., 2003).

The NF-κB-mediated antagonism of the death-inducing pathways triggered by TNFα depends, by and large, upon transcriptional upregulation of cytoprotective target genes (Kucharczak et al., 2003; Wajant et al., 2003). This seems to explain the early observation that unless inhibitors of RNA or protein synthesis are provided, cells usually resist the induction of PCD by TNFα (Kucharczak et al., 2003; Wajant et al., 2003). A handful of effectors of this protective activity of NF-κB have been identified and intensely investigated in recent years. These were shown to block the execution of TNFα-induced PCD through distinct molecular mechanisms (reviewed in Kucharczak et al., 2003; Wajant et al., 2003). Their best characterized examples are: cellular FLICE-inhibitory protein long (c-FLIPL), inhibiting early signal transduction events proximal to TNF-R1 and so prohibiting activation of caspase-8; the members of the Bcl-2 group of anti-apoptotic factors, A1/Bfl-1, Bcl-x_L, and Bcl-2, blocking PCD by preventing TNF-R-induced MOMP; cellular inhibitor of apoptosis proteins (c-IAP)1/2 and TRAF1/2, blunting apoptosis through a negative feedback regulation of receptor-triggered signaling, but apparently only when activated simultaneously; Spi2A, a newly-identified target of NF-κB, inhibiting TNFα-induced killing via a novel mechanism, entailing the suppression of cathepsin B, and so, of the lysosomal pathway of PCD (Liu et al., 2003).

The JNK MAPK pathway

The JNK pathway, also known as the stress activated protein kinase (SAPK) pathway, is one of the major mitogen activated protein kinase (MAPK) cascades mediating intracellular transduction of signals (Davis, 2000; Chang and Karin, 2001). Like the other two main MAPK cascades – the p38 and the extracellular-regulated kinases (ERK) cascades (Chang and Karin, 2001) – the JNK pathway transduces, amplifies and

integrates signals from a diverse array of cellular receptors in order to elicit an appropriate biological response. Signals are transmitted in these cascades through sequential phosphorylation events mediated by precisely regulated kinase modules (Davis, 2000; Chang and Karin, 2001). JNKs are encoded by the JNK1, JNK2 and JNK3 genes (Davis, 2000) and represent the terminal enzymatic module of the JNK cascade. In mammalian cells, kinases of the JNK group of MAPKs are primarily activated by proinflammatory cytokines, such as IL-1ß and TNFα, and stress stimuli such as UV radiation, pH changes, hypoxia, and genotoxic and oxidative stress (Davis, 2000). Activation of JNK in response to these stimuli has been linked to several biological responses, including proliferation, differentiation and apoptosis (Davis, 2000; Chang and Karin, 2001; Kennedy and Davis, 2003). By and large, however, induction of the JNK pathway, appears to be associated with the induction of PCD.

This involvement of JNK in PCD signaling is best illustrated by studies of mice harboring null mutations of JNK genes. *JNK1*$^{-/-}$ and *JNK2*$^{-/-}$ double knockout (dKO) MEFs are refractory to apoptosis induced by stress stimuli such as UV radiation, exhibiting impaired cytochrome c release and activation of caspase-3 in response to these stimuli (Tournier et al., 2000). Moreover, thymocytes lacking both JNK1 and JNK2 show a severe defect in the PCD response to the triggering of CD3 (Sabapathy et al., 2001), a component of the T-cell antigen receptor, *in vivo*. Finally, neurons from JNK3-/- mice are protected against killing induced by excitotoxins (Davis, 2000).

TNF-R-induced PCD signaling mediated by the JNK pathway

It is becoming increasingly clear that activation of the JNK cascade also plays an obligatory role in TNFα-induced PCD signaling. Several laboratories, including our own, have in fact reported that attenuation of this cascade is a pivotal means through which NF-κB blocks cytotoxicity triggered by the engagement of TNF-Rs (De Smaele et al., 2001; Javelaud and Besancon, 2001; Tang et al., 2001). Normally, TNFα only triggers transient activation of the JNK pathway. Following a rapid increase in the activity of JNK, occurring within minutes of the stimulation of TNF-Rs, this activity returns to basal levels usually within one hour (De Smaele et al., 2001; Javelaud and Besancon, 2001; Tang et al., 2001). However, upon inhibition of NF-κB by either knockout deletion of RelA or IKKß – the catalytic subunit of the IKK complex utilized by TNF-Rs (Hayden and Ghosh, 2004) – or overexpression of a degradation-resistant variant of IκBα, the shutdown of JNK signaling is severely compromised, thereby unveiling an additional sustained phase of this signaling downstream of TNF-Rs (De Smaele et al., 2001; Javelaud and Besancon, 2001; Tang et al., 2001). It is in fact this late, sustained phase of activation of JNK by TNFα that appears to be involved in mediating PCD signaling (De Smaele et al., 2001; Javelaud and Besancon, 2001; Tang et al., 2001; see also Papa et al., 2004a). In support of this view, persistent JNK activity induced by ectopic expression of MKK7-JNK1 proteins, mimicking constitutively active JNK, is sufficient alone to induce cell death (Lei et al., 2002). Thus, the NF-κB-mediated restraint of prolonged JNK activation is now generally viewed as a mandatory requirement for controlling PCD downstream of TNF-Rs (Papa et al., 2004a). Consistently, suppression of JNK activity by either treatment with pharmacological inhibitors or ectopic expression of dominant-negative JNK kinase mutants effectively rescues NF-κB null cells from TNFα-mediated cytotoxicity (De Smaele et al., 2001; Javelaud and Besancon, 2001; Tang et al., 2001). Death triggered by TNFα in these cells is also abrogated by siRNA-mediated silencing of MKK7 or compound genetic ablation of JNK1 and JNK2 (Deng et al., 2003; Ventura et al., 2004; Papa et al., 2004a). Interestingly, the inhibition of JNK in NF-κB-deficient cells can also block the necrosis-like response triggered by the engagement of TNF-Rs (Ventura et al., 2004) (see below).

It is worth noting that the inhibitory effects of NF-κB on the JNK cascade are unlikely to be an indirect consequence of suppressing activation of caspases, despite that these proteases are potentially capable of activating MAPKKKs (Davis, 2000). This is because, in NF-κB-deficient cells, JNK induction by TNFα remains sustained even after antiapoptotic treatment with the caspase inhibitor, z-VAD$_{fmk}$, (Javelaud and Besancon, 2001; Tang et al., 2001). Hence, compelling evidence in the literature now concurs with the notions that the TNFα-induced NF-κB and JNK pathways intersect and that a crucial outcome of this intersection is the inhibition of PCD.

Recent studies in animal models further highlight the biological relevance of this antagonistic crosstalk between the NF-κB and JNK pathways in promoting cell survival. It was shown that suppressing NF-κB activation in the liver of mice through conditional knockout mutation of IKKß leads to an exaggerated activation of JNK following systemic challenge with concanavalin A (ConA) (Maeda et al., 2003) – an agent that provokes hepatic damage through TNF-R-mediated signaling. It was also shown that this exaggerated induction of JNK correlates with the onset of liver injury and that targeted deletion of either *JKN1* or *JNK2* in IKKß-deficient mice markedly diminishes TNF-R-mediated injury triggered by ConA (Maeda et al., 2003). Thus, NF-κB suppresses the JNK pathway in order to prevent TNFα-induced hepato-toxicity, *in vivo*.

Mechanisms for JNK-mediated PCD signaling downstream of TNF-Rs

Exactly how JNK promotes PCD is not known. In some circumstances, JNK-mediated death signaling involves modulation of gene expression, achieved in part

via phosphorylation of the transcription factor, c-Jun (Davis, 2000). The main targets of TNFα-triggered cytotoxic JNK signaling, however, appear to be constitutively present in the cell (Davis, 2000). A recent report suggests a possible mechanism for the role of JNK in TNFα-inflicted cell death (Deng et al., 2003). The model envisions that activation of JNK by TNF-Rs provokes PCD by promoting the formation of jBid, a cleavage product of Bid that is generated through a caspase-8-independent mechanism and is distinct from tBid (Deng et al., 2003). Upon proteolytic processing, jBid seemingly migrates to mitochondria to trigger the selective release into the cytosol of the apoptogenic factor Smac/Diablo, but not of cytochrome c. Here, Smac/Diablo inhibits c-IAPs complexed to TRAF2, thereby causing activation of caspase-8 and, ultimately, PCD (Deng et al., 2003; Wajant et al., 2003) (Fig. 1). Consistent with this view, downregulation of Smac/Diablo by RNA-interference diminishes TNFα-induced activation of caspase-8 and PCD (Deng et al., 2003). The mechanism(s) by which JNK promotes formation of jBid, however, is not known. It is also unclear whether Bid is the only target of proapoptotic JNK signaling, as there might be other means through which JNK promotes TNFα-triggered cytotoxicity. Indeed, killing by overexpressed MKK7-JNK1 chimeric proteins was shown to require Bax-like factors of the Bcl-2 group, but not Bid – inferred from the normal activity of these chimeric proteins in Bid-/- cells (Lei et al., 2002). Hence, "BH-3-only" factors other than Bid might participate in JNK-mediated killing in this system. JNK is also capable of triggering necrosis-like PCD (Papa et al., 2004a; Ventura et al., 2004); yet, the means by which JNK inflicts TNFα-induced necrosis is not known.

Interestingly, despite the crucial role of JNK in

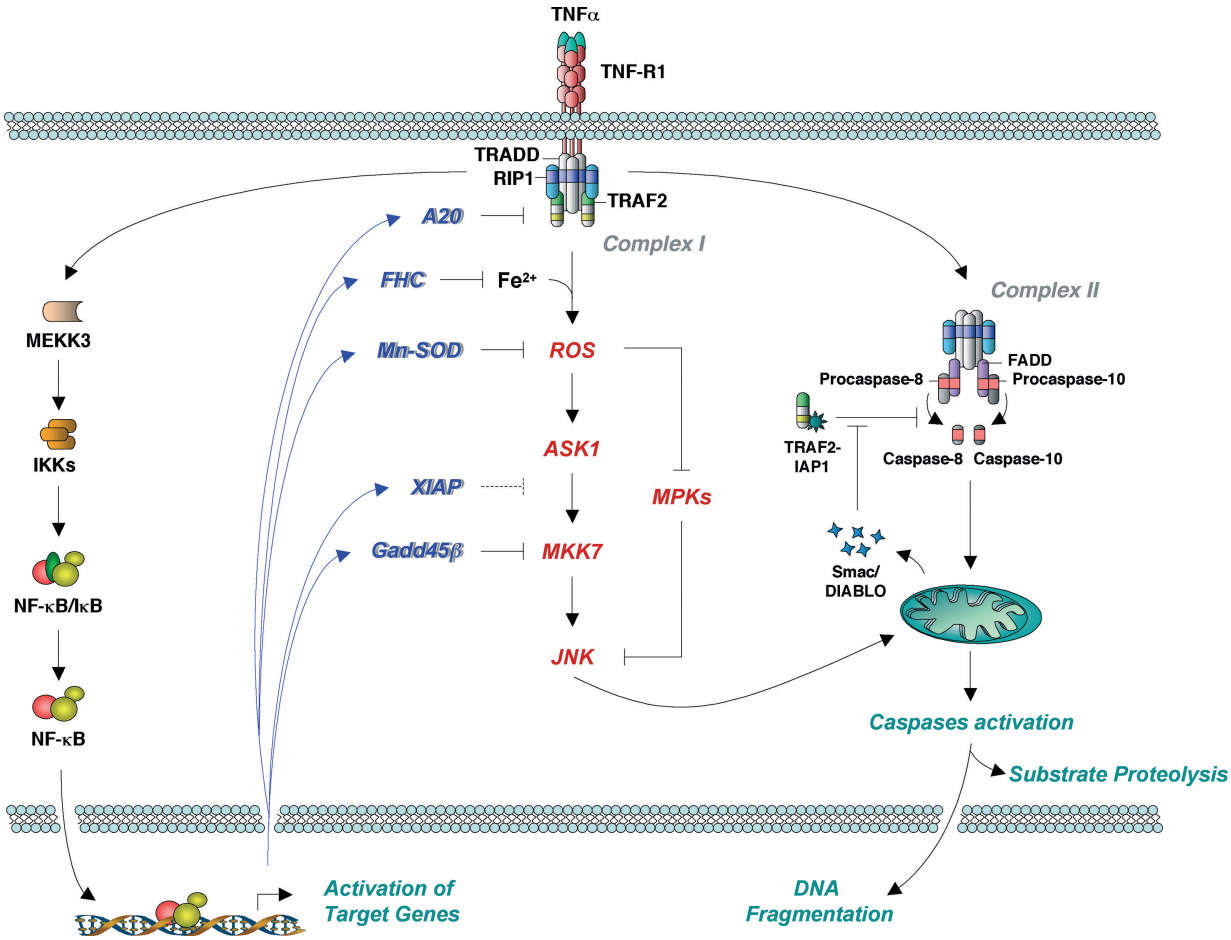

Fig. 1. Mechanisms behind the anti-apoptotic activity of NF-κB in ROS/JNK signaling. The accumulation of ROS upon engagement of TNF-R1 by TNFα promotes cell death through activation of the JNK MAPK cascade. This occurs via at least two mechanism: inhibition of MKPs and activation of MAPKKKs such as ASK1. Death signaling triggered by ROS-mediated activation of JNK is effectively antagonized by NF-κB, which upregulates expression of protective genes such as FHC, Mn-SOD, A20, Gadd45ß and XIAP, blocking the ROS/JNK pathway.

TNFα-induced PCD, cell death does not appear to be the only possible outcome of activation of JNK. In some systems, JNK induction might in fact even be a signal for cell survival (Lamb et al., 2003; Reuther-Madrid et al., 2002). In a recent study, fibroblasts from *JNK1-/- JNK2-/-* embryos were found to be highly sensitive to apoptosis induced by stimulation with TNFα, suggesting that in the presence of functional NF-κB complexes, activation of the JNK pathway might serve to facilitate cell survival (Lamb et al., 2003). In a subsequent study, also utilizing JNK dKO MEFs, the same group reported that, while blocking apoptosis, JNK is a key participant in necrosis-like PCD induced by TNF-Rs (Ventura et al., 2004). Nevertheless, the net result of activating JNK following stimulation of TNF-Rs appears to be an overall increase in cell death (Ventura et al., 2004). The basis for the apparent discrepancies between some of the studies present in the literature is not clear. Most of these studies, however, seem consistent with the notion that the biological outcome of JNK activation ultimately depends also upon stimulus- and tissue-specific factors involving crosstalk and integration of JNK signaling with other pathways that might be concomitantly active in the cell, such as the ERK and the Akt/PKB pathways (Davis, 2000; Papa et al., 2004a). It is also likely that prosurvival signaling involves a transient activation of JNK, perhaps affecting gene expression (Lamb et al., 2003), and that the role of JNK in TNFα-mediated PCD relies instead upon this activation being sustained (Davis, 2000; Papa et al., 2004a).

NF-κB-inducible inhibitors of the JNK pathway

The primary mechanism through which NF-κB secures an effective shutdown of the JNK pathway following the triggering of TNF-Rs seems to involve activation of select downstream targets (Kucharczak et al., 2003; Wajant et al., 2003; Papa et al., 2004a) (Fig. 1). Few such targets of NF-κB that encode *bona fide* blockers of the JNK pathway have been identified. The best characterized of these are the zinc-finger protein A20, the Gadd45-family member Gadd45ß/Myd118 and the c-IAP-like factor, XIAP (Kucharczak et al., 2003; Papa et al., 2004a).

Upon ligation of TNF-Rs, transcription of A20 is rapidly induced by NF-κB (Boone et al., 2002; Kucharczak et al., 2003). This induction seems to play a critical role in the NF-κB-mediated downregulation of the JNK cascade, because MEFs from $A20^{-/-}$ mice display sustained activation of JNK and an exaggerated apoptotic response following the triggering of TNF-Rs (Lee et al., 2000; Boone et al. 2002). Interestingly, however, ectopic expression of A20 fails to attenuate PCD in NF-κB-deficient cells (Beg and Baltimore, 1996; Kucharczak et al., 2003). Thus, whereas A20 appears to be an essential mediator of the prosurvival activity of NF-κB, this factor alone is insufficient to fully explain the inhibitory action of NF-κB on JNK and PCD signaling downstream of TNF-Rs. Furthermore, the mechanism(s) by which A20 hampers the JNK cascade remains unclear. A20 is a key player in the negative feedback mechanism responsible for terminating NF-κB activation during TNFα-triggered signal transduction (Lee et al., 2000; Boone et al., 2002). This downregulation of the NF-κB pathway by A20 was reported to involve A20-mediated ubiquitination and inactivation of RIP1 (Wertz et al., 2004), a factor needed for activation of NF-κB by TNF-Rs (Wajant et al., 2003). RIP1, however, does not participate in the TNF-R-triggered induction of JNK (Wajant et al., 2003), and so, the A20-mediated inhibition of the JNK pathway is probably owed to a different mechanism. One possibility is that A20 blocks TNFα-induced JNK signaling through inactivation of TRAF2 – a molecule required for activation of JNK by TNFα (Davis, 2000) – or of another molecule residing proximal to TNF-R1 (Boone et al., 2002; Wajant et al., 2003). Indeed, A20 is capable of physically interacting with TRAF2 (Boone et al., 2002) and is recruited to the TNF-R1 complex upon engagement of this receptor by TNFα (Zhang et al., 2000). Nevertheless, determining the precise mechanism for how A20 mediates downregulation of the JNK cascade requires further investigation.

Work from our own laboratory previously showed that *Gadd45ß*, originally identified as an immediate-early gene induced by TGFß (Liebermann and Hoffmann, 2002), is another key component of the transcriptional mechanism by which NF-κB contains the JNK cascade (De Smaele et al., 2001). Gadd45ß is a member of the Gadd45 family of related factors, also including Gadd45α and Gadd45γ (Liebermann and Hoffmann, 2002). Members of this family (and in particular Gadd45α) have been implicated in multiple processes, including the control of cell cycle, DNA repair and the regulation of MAPKs (Takekawa and Saito, 1998; De Smaele et al., 2001; Liebermann and Hoffmann, 2002; Hollander and Fornace, 2002; Papa et al., 2004a). Unlike other Gadd45-family genes, Gadd45ß is upregulated by NF-κB in response to treatment with TNFα (De Smaele et al., 2001). Moreover, overexpression of Gadd45ß in NF-κB null cells virtually abrogates TNFα-induced activation of JNK signaling (De Smaele et al., 2001). Most importantly, inactivation of endogenous Gadd45ß impairs the downregulation of JNK activity induced by TNFα (De Smaele et al., 2001), indicating that upregulation of Gadd45ß is an obligatory requirement for the NF-κB-mediated control of this activity. Direct genetic evidence for an essential role of Gadd45ß in the NF-κB-mediated containment of TNFα-induced JNK signaling was also obtained with the analysis of fibroblasts from Gadd45ß-/- mice (Papa et al., 2004b). Nevertheless, work from another laboratory suggests that knockout mutation of Gadd45ß in MEFs has no effect on PCD induced by TNFα (Amanullah et al., 2003). The basis for this apparent discrepancy is presently unknown. It is likely, however, that it is due, at least in part, to the different experimental conditions used in these two studies (further discussed in Zazzeroni

et al. 2003).

In a recent report, we have shown that the Gadd45ß-mediated suppression of JNK signaling involves the direct targeting and inhibition of MKK7/JNKK2 (Papa et al 2004b), the dominant JNK kinase induced by TNFα (Davis, 2000; Tournier et al., 2001). Of interest, preliminary in vitro experiments suggest that Gadd45ß blocks activity of this kinase by preventing its access to ATP (Papa et al., 2004b). Consistent with the notion that Gadd45ß is an important downstream effector of the inhibitory activity of NF-κB on the JNK cascade, NF-κB-deficient cells fail to properly shutdown activation of MKK7 during stimulation with TNFα (Papa et al., 2004b). Hence, the interaction between Gadd45ß and MKK7 represents a crucial molecular link between the NF-κB and JNK pathways. Indeed, although Gadd45ß has the ability to interact with other kinases in the JNK pathway, blockade of MKK7 alone seems to adequately explain the inhibitory effects of Gadd45ß on this pathway (discussed in Papa et al., 2004a).

Another mechanism by which NF-κB has been proposed to control the JNK pathway involves upregulation of XIAP (Kucharczak et al., 2003), a member of the IAP family of inhibitors of caspases (Salvesen and Duckett, 2002). XIAP is also a known target of NF-κB, and its overexpression in NF-κB-deficient cells attenuates cytotoxicity induced by TNFα (Stehlik et al., 1998). Indeed, thymocytes of XIAP transgenic mice are refractory to PCD triggered by various stimuli (Salvesen and Duckett, 2002). Moreover, ectopic expression of XIAP in RelA null cells blunts activation of the JNK cascade downstream of TNF-Rs, but seems to have no effect on p38 and ERK signaling (Tang et al., 2001). Notably, XIAP is capable of inhibiting both the caspase-dependent and the caspase-independent phases of JNK activation by TNFα (Tang et al., 2001). Thus, the effects of XIAP on MAPK signaling are unlikely to be solely due to its ability to suppress activity of caspases (Salvesen and Duckett, 2002). Nevertheless, the precise mechanism for the XIAP-mediated inhibition of the JNK cascade remains unknown. In fact, whereas XIAP is apparently capable of associating with kinases within the JNK pathway, these interactions of XIAP have been shown to promote (rather than inhibit) downstream activation of JNK (Salvesen and Duckett, 2002; Sanna et al., 2002). Finally, XIAP-/- mice display no apparent defect in JNK or PCD signaling in response to the triggering of TNF-Rs (Harlin et al., 2001; Kucharczak et al., 2003). Thus, further studies are required to establish the physiological relevance of the XIAP-mediated regulation of the JNK cascade.

Transduction of TNF-R-induced PCD signaling by ROS

Notably, recent studies have unveiled another key mechanism by which NF-κB blocks cytotoxicity triggered by TNFα, namely the suppression of the accumulation of reactive oxygen species (ROS) (Sakon et al., 2003; Pham et al., 2004). ROS are in fact emerging as obligatory mediators of cell death signaling induced in response to stimulation of TNF-Rs (Sakon et al., 2003; Pham et al., 2004; Ventura et al., 2004; Kamata et al., 2005). Compared to their wild-type counterparts, NF-κB-deficient cells exhibit an abnormal accumulation of ROS upon the engagement of these receptors by TNFα (Sakon et al., 2003; Pham et al., 2004; Ventura et al., 2004), and suppression of this accumulation by antioxidant agents affords near-complete protection against TNF-R-induced killing in these cells (Sakon et al., 2003; Pham et al., 2004). Thus, ROS appear to be at a crucial crossroad of the death-inducing and pro-survival pathways elicited by the triggering of TNF-Rs. Accordingly, they have now taken center stage within the complex interplay of mechanisms controlling cell fate downstream of these receptors. The potent reactivity of ROS appears in fact to have been exploited also by other pathways capable of inflicting cell death, including the pathways initiated by radiation and chemotherapeutic drugs (Curtin et al., 2002).

The precise means by which TNF-Rs induces formation of ROS are presently unknown. It is normally assumed that TNFα-induced ROS are primarily produced in mitochondria – the main source of oxygen radicals in eukaryotes (Curtin et al., 2002). However, prior studies have not conclusively addressed this issue, because these studies have generally measured TNF-R-dependent ROS formation at rather late times (i.e. several hours), and so cannot differentiate between the ROS acting as second messengers in signal transduction and those being produced as a consequence of the oxidative burst that invariably follows MOMP, a sign that cell might have already committed to die (Wajant et al., 2003). Thus, whether mitochondrial ROS are a cause or a secondary effect of cell death remains unclear. Indeed, the previous observations that ROS formation is not induced by the triggering of IL-1ß-R and that this formation downstream of TNF-R1 can be blocked by knockout ablation of JNK1 and JNK2 might simply reflect an absence of cell death in these systems (Sakon et al., 2003; Ventura et al., 2004; Kamata et al., 2005). The view that mitochondria are the primary source of TNFα-stimulated ROS is also somewhat at odds with the relatively weak protective activity of overexpression of the mitochondrial antioxidant enzyme, Mn^{++} superoxide dismutase (Mn-SOD) (Sakon et al., 2003; Pham et al., 2004). Extra-mitochondrial sources of ROS have in fact been identified and proposed to play a role in induction of PCD by TNF-R1 (Jaattela and Tschopp 2003). Thus, the issue of which is the precise origin of ROS generated downstream of TNF-Rs requires further investigation and the employment of new, more sophisticated methods for their detection.

The bases for the ROS-mediated activation of JNK signaling downstream of TNF-Rs

As it turns out, ROS-inflicted cytotoxicity downstream of TNF-Rs depends in part on the induction

of sustained JNK activity (Pham et al., 2004; Kamata et al., 2005; Matsuzawa and Ichijo, 2005). Thus, the activities of ROS and JNK appear to participate in the same death-inducing mechanism activated by stimulation of TNF-Rs. The antioxidant action of NF-κB represents in fact a pivotal mechanism through which NF-κB restrains sustained activation of the JNK pathway by TNFα (Sakon et al., 2003; Pham et al., 2004) (Fig. 1). Recent studies are now beginning to elucidate how ROS promote the induction of JNK signaling. One such study has identified phosphatases of the MAP kinase phosphatase (MKP) group (Davis, 2000), including MKP-1, -3-, -5 and -7, as critical molecular targets of ROS during TNFα-induced PCD (Kamata et al., 2005). ROS-mediated inactivation of MKPs – which play a critical role in the regulation of the activity of MAPKs (Davis, 2000) – seemingly involves oxidation of a cysteine residue within the catalytic domain of these enzymes to sulfenic acid (Kamata et al., 2005). The physiological relevance of this mechanism is supported by the finding that this residue exhibits a lower pKa than other, non-catalytic cysteine residues of MKPs (Kamata et al., 2005), and so is more susceptible to oxidation than these other cysteine residues. Thus, ROS-dependent inhibition of MKPs causes persistent activation of JNK by TNFα and, ultimately, PCD via either a necrotic or an apoptotic pathway (Kamata et al., 2005). Accordingly, treatment with antioxidants virtually abrogates sustained activation of the JNK pathway and both necrosis and apoptosis caused by the triggering of TNF-Rs. These conclusions, however, await confirmation through genetic means.

The inactivation of MKP phosphatases does not appear to be the only mechanism for the ROS-mediated activation of sustained JNK signaling downstream of TNF-Rs. Previous studies have in fact shown that ROS also promote activation of ASK1/MEKK5, a TRAF2-binding MAPK kinase kinase (MAPKKK) involved in prolonged induction of JNK and PCD by TNF-R1 (Tobiume et al., 2001; Matsuzawa and Ichijo, 2005). This model is supported by knockout data, since $ASK1^{-/-}$ fibroblasts exhibit a profound defect in the induction of sustained JNK signaling and apoptosis in response to stimulation with TNFα (Tobiume et al., 2001). Interestingly, as reported for the inactivation of MKPs (Kamata et al., 2005), ROS-mediated induction of ASK1 appears to depend upon inhibition of the redox-sensing factor, thioredoxin (Liu et al., 2000; Matsuzawa and Ichijo, 2005). Thus, both the amplitude and duration of TNFα-induced JNK signaling are seemingly dictated by a balance between the activities of inducing kinases and those of inhibiting phosphatases. Most likely, the relative importance of these opposing activities for induction of JNK signaling ultimately depends upon the biological context. The precise definition of the MAPKKKs that are involved in the acute and persistent phases of JNK activation by TNF-Rs will help clarify the bases for the ROS-mediated regulation of the JNK cascade.

Most available studies seem to concur that ROS lie upstream of JNK in the TNF-R-triggered pathway of PCD (Sakon et al., 2003; Pham et al., 2004; Kamata et al., 2005). However, some evidence indicates that there might be in this pathway a more complex crosstalk between ROS and JNK activities. In fact, it was recently found that the accumulation of ROS normally observed in NF-κB-deficient fibroblasts after treatment with TNFα is severely compromised by concomitant knockout deletion of JNK1 and JNK2 (Ventura et al., 2004), suggesting that in these cells TNF-R1-triggered JNK activity is required for downstream induction of ROS. Hence, JNK and ROS might participate in a positive feedback loop, and this loop might be what is ultimately responsible for induction of cell death (Ventura et al., 2004). It should be cautioned, though, that while a dynamic relationship between ROS and JNK is likely to exist, it is possible that the antioxidant effects of ablating JNK genes in this system also depends upon the suppression of cell death.

Interestingly, the actual ordering of the inductions of ROS and JNK might dictate how a cell dies in response to the triggering of TNF-Rs (i.e. via necrosis or apoptosis). TNFα is in fact capable of eliciting both the apoptotic and the necrotic (i.e. caspase-independent) pathways of PCD (Sakon et al., 2003, Pham et al., 2004, Ventura et al., 2004; Kamata et al., 2005), and so, based on the aforementioned report (Ventura et al., 2004), it is possible that in the TNF-R1-triggered pathway of necrosis ROS lie downstream (rather than upstream) of the activation of JNK. Which form of cell death is predominantly activated following stimulation of TNF-Rs is also likely to be determined by the specific cell system and experimental conditions that are used. Yet, despite that the precise relationship between ROS and JNK signaling, as well as the biological consequences of this relationship, remain to be defined, virtually all studies seem to agree that activation of NF-κB is capable of counteracting TNFα-triggered cytotoxicity regardless of the specific pathway of PCD that is induced downstream of TNF-Rs (Sakon et al., 2003, Pham et al., 2004, Ventura et al., 2004). Further, irrespective of their relative positioning with respect to JNK, ROS are now being recognized as obligatory mediators in the death-inducing mechanism triggered by TNFα (Sakon et al., 2003; Pham et al., 2004; Ventura et al., 2004; Kamata et al., 2005).

Mechanisms for the NF-κB-mediated suppression of ROS

The importance of ROS generation in TNF-R-induced PCD signaling is also underscored by the finding that this generation is tightly controlled by NF-κB (Sakon et al., 2003; Pham et al., 2004). The bases for the NF-κB-dependent restraint of redox disequilibrium are now beginning to be unveiled. So far, at least two downstream targets have been proposed to mediate this important function of NF-κB. Using a gene array-based screen, we have identified Ferritin heavy chain (FHC) as

a pivotal effector of the antioxidant and protective actions of NF-κB downstream of TNF-Rs (Pham et al., 2004). Together with light chains (FLC), FHC forms Ferritin complexes, the primary iron storage mechanism found in cells (Torti and Torti, 2002). FHC is upregulated by TNFα through a mechanism controlled by NF-κB, is required for inhibition of TNFα-induced killing, and blocks PCD in NF-κB-deficient cells (Pham et al., 2004). The FHC-mediated blockade of PCD involves attenuation of ROS accumulation in response to TNF-R stimulation, and this attenuation prevents sustained activation of the JNK cascade (Pham et al., 2004). The antioxidant activity of FHC depends on sequestration of free iron (Pham et al., 2004) – a transition metal that catalyzes the formation of ROS in mitochondria and through Fenton reaction, resulting in production of highly reactive hydroxyl (•OH) radicals (Torti and Torti, 2002). Notably, gene-silencing experiments suggest that, in certain tissues, FHC may constitute a pivotal component of the protective mechanism activated by NF-κB for controlling TNF-R-induced JNK signaling and PCD (Pham et al., 2004).

FHC is one of several acute-phase proteins, induced in the liver during the organismal response to stress, injury and infection (Torti and Torti, 2002). Through this induction of FHC, NF-κB may promote hypoferremia, and so, restrict iron availability in peripheral tissues during chronic inflammation, when the potential exists for causing extensive ROS-mediated tissue damage (Makarov, 2000; Tak and Firestein, 2001). Indeed, this may represent a systemic cytoprotective mechanism that is mediated by NF-B. Notably, FHC might also play a prominent role in NF-κB-dependent oncogenesis, tumor progression and cancer chemo- and radio-resistance (Torti and Torti, 2002). High levels of FHC have in fact been found in several tumors and have been associated with resistance to anti-cancer treatment and an aggressive malignant phenotype (Torti and Torti, 2002).

Another factor that has been proposed to play an important role as effector of the ROS-inhibiting and cytoprotective actions of NF-κB is Mn-SOD (Bernard et al., 2001; Delhalle et al., 2002; Kucharczak et al., 2003; Wajant et al., 2003) – an enzyme that catalyzes dismutation of superoxide anion ($\bullet O_2^-$) into hydrogen peroxide (H_2O_2) (Curtin et al., 2002). Mn-SOD is upregulated by TNFα through a transcriptional mechanism that requires NF-κB and can blunt TNFα-induced cytotoxicity in certain systems (Bernard et al., 2001; Delhalle et al., 2002; Kucharczak et al., 2003; Pham et al., 2004). Yet, the significance of Mn-SOD to the protective mechanism activated by NF-κB against ROS-inflicted tissue damage remains uncertain. This is because, in NF-κB-deficient cells, ectopic expression of Mn-SOD seems to afford little or no protection against TNFα-induced killing (Sakon et al., 2003; Pham et al., 2004). Moreover, in some tissues neither basal nor TNFα-activated levels of Mn-SOD appear to be controlled by NF-κB (Delhalle et al., 2002; St. Clair et al., 2002; Pham et al., 2004). Nevertheless, despite that it might be insufficient alone to effectively block ROS accumulation and PCD triggered by TNF-Rs and that it might mediate the NF-κB protective action only in certain tissues, Mn-SOD might still be required for the control of TNFα-prompted changes of cellular redox status. Indeed, for an effective suppression of ROS, the synergistic activities of FHC and Mn-SOD might be key. Indeed, it is conceivable that whereas induction of Mn-SOD promotes dismutation of $\bullet O_2^-$ into H_2O_2, FHC-mediated sequestration of iron permits disposal of H_2O_2 by peroxidases and catalases.

The coordinate upregulations of the antioxidant genes, *Mn-SOD* and *FHC*, seem to provide also an additional link between the NF-κB and JNK pathways activated in response to stimulation of TNF-Rs (Fig. 1). Hence, NF-κB appears to halt the JNK cascade through two distinct mechanisms: directly, through an induction of A20, Gadd45ß and XIAP (Papa et al., 2004a); and indirectly, through an induction of FHC and Mn-SOD – which together restrain accumulation of ROS (Fig. 1) (Bubici et al., 2004). Most likely, to ensure an effective shutdown of proapoptotic JNK signaling, these factors have to act cooperatively. Nevertheless, the biological relevance of the specific mechanism for JNK inhibition that is activated by NF-κB likely depends upon tissue and biological context. Indeed, the adaptability of the NF-κB-inducible program of JNK inhibition might allow an organism to orchestrate an appropriate prosurvival response to each apoptotic stimulus and biological context.

Relevance to the treatment of human diseases and future directions

Disturbances of the NF-κB-mediated suppression of TNF-R-induced PCD play an important role in widespread human diseases (Makarov et al., 2000; Orlowski and Baldwin, 2002; Kucharczak et al., 2003). Indeed, the positive feedback regulation existing between TNFα and NF-κB represents a critical pathogenetic mechanism perpetuating chronic inflammation in conditions such as RA and IBD (Makarov, 2000; Tak and Firestein, 2001; Kucharczak et al., 2003). Accordingly, current treatments for these conditions include drugs that inhibit either NF-κB, such as non-steroidal anti-inflammatory drugs (NSAIDs) and glucocorticoids, or TNFα, such as anti-TNFα neutralizing antibodies (Makarov, 2000; Tak and Firestein, 2001; Kucharczak et al., 2003; Karin et al., 2004). Likewise, glucocorticoids and proteasome inhibitors, which also block the NF-κB pathway, have been used successfully to treat human malignancies such as HL and MM (Orlowski and Baldwin, 2002; Kucharczak et al., 2003; Karin et al., 2004). However, the clinical applicability of these therapies has been severely limited by their often serious side-effects – most notably, the immunosuppressive effects. A radical new approach to therapy is therefore needed. For instance, to avoid harming the immune system, such

approach should enable the selective targeting of the downstream protective effectors of NF-κB, rather than NF-κB itself. Remarkably, this goal has now become realistic, as it is evident at this point that – while integrated – the actions of NF-κB in immunity and cell survival are executed through independent subsets of targets genes.

Accordingly, an important future challenge is identifying the effectors and precise mechanisms by which NF-κB directs attenuation of ROS accumulation and JNK signaling in specific patho-physiological contexts. This might in fact lead to the development of new drugs that selectively interfere with the crosstalk between the NF-κB and ROS/JNK pathways in these contexts, and so, promote PCD in diseased tissues without seriously impacting on the ability of NF-κB to function in immunity (Kucharczak et al., 2003). For instance, an augmentation of ROS and/or JNK signaling, achieved through inhibition of select targets of NF-κB, might trigger PCD in proinflammatory cells within inflamed tissues – where there are high concentrations of TNFα (Makarov, 2000; Tak and Firestein, 2001). The emerging notion that the program activated by NF-κB to suppress the inductions of ROS and JNK signaling has tissue- and context-specific components (Papa et al., 2004a) suggests that it might be possible to further increase selectivity of these drugs for specific tissues. Illustrating this point is a recent study showing that the ROS-mediated activation of JNK signaling in the liver is selectively involved in concanavalin-A-inflicted injury, but not in parenchymal regeneration following partial hepatectomy, albeit both processes are governed by an integration of the activities of TNF-Rs, JNK and NF-κB (Kamata et al., 2005).

In addition to providing important new targets for anti-inflammatory therapy, these studies might prove crucial for developing new approaches for treatment of cancer. In many tumors, JNK (and ROS) and NF-κB seem in fact to have opposing biological effects. Whereas NF-κB activation is required to block transformation-associated PCD induced by oncoproteins such as H-Ras(V12) and Her-2/Neu (Kucharczak et al., 2003; Orlowski and Baldwin, 2002), these oncoproteins are known to be potent inducers of JNK (Davis, 2000; Kennedy and Davis, 2003). Moreover, NF-κB promotes survival of certain late-stage cancers and antagonizes cytotoxicity triggered by radiation and chemotherapeutic drugs such as topoisomerase inhibitors (Orlowski and Baldwin, 2002; Kucharczak et al., 2003). In contrast, the inductions of JNK and/or ROS mediate cancer cell killing induced by some anti-cancer agents, and activators of the JNK pathway (such as JNK3, MKK4 and BRCA1) have been found to act as tumor suppressors (Davis, 2000; Kennedy and Davis, 2003). Thus, an augmentation of ROS or JNK signaling, induced for instance through a blockade of select NF-κB targets, might represent a powerful new line of attack for anti-cancer treatment.

Concluding remarks

It is now becoming apparent that, while integrated, the actions of NF-κB in immunity and cell survival are executed through induction of distinct subsets of target genes. The restraint that NF-κB imposes on ROS accumulation and JNK signaling is critical for several physiological processes, as well as for chronic inflammation and cancer. In recent years, the identification of several NF-κB-inducible inhibitors of the JNK cascade and ROS formation has advanced our understanding for how this restraint is mediated. The redundancy reflected in having multiple effectors for these activities of NF-κB may be crucial for ensuring an effective shutdown of ROS accumulation and JNK signaling. Further, redundancy might allow an organism to modulate the prosurvival response according to specific biological contexts and needs. Undoubtedly, it would be of crucial interest to establish the precise role(s) of individual NF-κB-inducible genes in each of these contexts and determine exactly how their products inhibit ROS formation and/or activation of the JNK cascade. Since inappropriate blockade of PCD by NF-κB appears to be a key pathogenetic element in widespread human diseases, these endeavors will likely facilitate the development of novel, highly selective approaches for treatment of these diseases.

Acknowledgements. We thank Gita Kapila for help with manuscript preparation. This research was supported in part by NIH grants R01-CA84040 and R01-CA098583 and grant from the Cancer Research Institute.

References

Alcamo E., Mizgerd J.P., Horwitz B.H., Bronson R., Beg A.A., Scott M., Doerschuk C.M., Hynes R.O. and Baltimore D. (2001). 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.

Amanullah A., Azam N., Balliet A., Hollander C., Hoffman B., Fornace A. and Liebermann D. (2003). Cell signalling: cell survival and a Gadd45-factor deficiency. Nature 424, 741.

Amit S. and Ben-Neriah Y. (2003). NF-κB activation in cancer: a challenge for ubiquitination- and proteasome-based therapeutic approach. Semin Cancer Biol. 13, 15-28.

Beg A.A. and Baltimore D. (1996). An essential role for NF-κB in preventing TNFα-induced cell death. Science 274, 782-784.

Beg A.A., Sha W.C., Bronson R.T., Ghosh S. and Baltimore D. (1995). Embryonic lethality and liver degeneration in mice lacking the RelA component of NF-κB. Nature 376, 167-170.

Bernard D., Quatannens B., Begue A., Vandenbunder B. and Abbadie C. (2001). Antiproliferative and antiapoptotic effects of crel may occur within the same cells via the up-regulation of manganese superoxide dismutase. Cancer Res. 61, 2656-2664.

Boone D.L., Lee E.G., Libby S., Gibson P.J., Chien M., Chan F., Madonia M., Burkett P.R. and Ma A. (2002). Recent advances in

understanding NF-κB regulation. Inflam. Bowel Dis. 8, 201-212.

Bubici C., Papa S., Pham C.G., Zazzeroni F. and Franzoso G. (2004). NF-κB and JNK: an intricate affair. Cell Cycle 3, 1524-1529.

Chang L. and Karin M. (2001). Mammalian MAP kinase signalling cascades. Nature 410, 37-40.

Chen L.F. and Greene W.C. (2004). Shaping the nuclear action of NF-κB. Nat. Rev. Mol. Cell. Biol. 5, 392-401.

Curtin J.F., Donovan M. and Cotter T.G. (2002). Regulation and measurement of oxidative stress in apoptosis. J. Immunol. Methods 265, 49-72.

Danial N.N. and Korsmeyer S.J. (2004). Cell death: critical control points. Cell 116, 205-219.

Davis R.J. (2000). Signal transduction by the JNK group of MAP kinases. Cell 103, 239-252.

De Smaele E., Zazzeroni F., Papa S., Nguyen D.U., Jin R., Jones J., Cong R. and Franzoso G. (2001). Induction of gadd45ß by NF-κB downregulates pro-apoptotic JNK signalling. Nature 414, 308-313.

Delhalle S., Deregowski V., Benoit V., Merville M.P. and Bours V. (2002). NF-κB-dependent MnSOD expression protects adenocarcinoma cells from TNF-α-induced apoptosis. Oncogene 21, 3917-3924.

Deng Y., Ren X., Yang L., Lin Y. and Wu X. (2003). A JNK-dependent pathway is required for TNFα-induced apoptosis. Cell 115, 61-70.

Doi T.S., Marino M.W., Takahashi T., Yoshida T., Sakakura T., Old L. J. and Obata Y. (1999). Absence of tumor necrosis factor rescues RelA-deficient mice from embryonic lethality. Proc. Natl. Acad. Sci. USA 96, 2994-2999.

Gerondakis S. and Strasser A. (2003). The role of Rel/NF-κB transcription factors in B lymphocyte survival. Semin Immunol. 15, 159-66.

Ghosh S. and Karin M. (2002) Missing pieces in the NF-κB puzzle. Cell 109 Suppl: S81-96.

Harlin H., Reffey S.B., Duckett C.S., Lindsten T. and Thompson C.B. (2001). Characterization of XIAP-deficient mice. Mol. Cell. Biol. 21, 3604-3608.

Hayden M.S. and Ghosh S. (2004). Signaling to NF-κB. Genes Dev. 18, 2195-2224.

Hollander M.C. and Fornace A.J. (2002). Genomic instability, centrosome amplification, cell cycle checkpoints and Gadd45α. Oncogene 21, 6228-6233

Jaattela M. and Tschopp J. (2003). Caspase-independent cell death in T lymphocytes. Nat. Immunol. 4, 416-423.

Javelaud D. and Besancon F. (2001). NF-κB activation results in rapid inactivation of JNK in TNF alpha-treated Ewing sarcoma cells: a mechanism for the anti-apoptotic effect of NF-κB. Oncogene 20, 4365-4372.

Johnstone R.W., Ruefli AA. and Lowe S.W. (2002). Apoptosis: a link between cancer genetics and chemotherapy. Cell 108, 153-164.

Kamata H., Honda S., Maeda S., Chang L., Hirata H. and Karin M. (2005). Reactive oxygen species promote TNFα-induced death and sustained JNK activation by inhibiting MAP kinase phosphatases. Cell 120, 649-661.

Karin M. and Ben-Neriah Y. (2000). Phosphorylation meets ubiquitination: the control of NF-κB activity. Annu. Rev. Immunol. 18, 621-663.

Karin M. and Lin A. (2002). NF-κB at the crossroads of life and death. Nat. Immunol. 3, 221-227.

Karin M., Yamamoto Y. and Wang Q.M. (2004). The IKK NF-κB system: a treasure trove for drug development. Nat. Rev. Drug Discov. 3, 17-26.

Kennedy N.J. and Davis R.J. (2003). Role of JNK in tumor development. Cell Cycle 2, 199-201.

Kucharczak J., Simmons M.J., Fan Y. and Gelinas C. (2003). To be, or not to be: NF-κB is the answer-role of Rel/NF-κB in the regulation of apoptosis. Oncogene 22, 8961-8982.

Lamb J.A., Ventura J.J., Hess P., Flavell R.A. and Davis R.J. (2003). JunD mediates survival signaling by the JNK signal transduction pathway. Mol. Cell 11, 1479-1489.

Lee E.G., Boone D.L., Chai S., Libby S., Chien M., Lodolce J.P. and Ma A. (2000). Failure to regulate TNF-induced NF-κB and cell death responses in A20-deficient mice. Science 289, 2350-2354.

Lei K., Nimnual A., Zong W.X., Kennedy N.J., Flavell R.A., Thompson C.B., Bar-Sagi D. and Davis R.J. (2002). The Bax subfamily of Bcl2-related proteins is essential for apoptotic signal transduction by c-Jun NH(2).-terminal kinase. Mol. Cell Biol. 22, 4929-4942.

Li Q. and Verma I.M. (2002). NF-κB regulation in the immune system. Nat. Rev. Immunol. 2, 725-734.

Liebermann D.A. and Hoffmann B. (2002). Myeloid differentiation (MyD) primary response genes in hematopoiesis. Oncogene 21, 3391-3402.

Liu H., Nishitoh H., Ichijo H. and Kyriakis J.M. (2000). Activation of apoptosis signal-regulating kinase 1 (ASK1) by tumor necrosis factor receptor-associated factor 2 requires prior dissociation of the ASK1 inhibitor thioredoxin. Mol. Cell. Biol. 20, 2198-2208.

Liu N., Raja S., Zazzeroni F., Metkar S.S., Shah R., Zhang M., Wang Y., Bromme D., Russin W.A., Lee J.C., Peter M.E., Froelich C., Franzoso G. and Ashton-Rickardt P.G. (2003). NF-κB protects from the lysosomal pathway of cell death. EMBO J. 22, 5313-5322.

Liu Z.G., Hsu H., Goeddel D. and Karin M. (1996). 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.

Maeda S., Chang L., Li Z.W., Luo J.L., Leffert H. and Karin M. (2003). IKKß is required for prevention of apoptosis mediated by cell-bound but not by circulating TNFalpha. Immunity 19, 725-737.

Makarov S.S. (2000). NF-κB as a therapeutic target in chronic inflammation: recent advances. Mol. Med. Today 6, 441-448.

Matsuzawa A. and Ichijo H. (2005). Stress-responsive protein kinases in redox-regulated apoptosis signaling. Antioxid Redox Signal. 7, 472-481.

Micheau O. and Tschopp J. (2003). Induction of TNF receptor I-mediated apoptosis via two sequential signaling complexes. Cell 114, 181-90.

Orlowski R.Z. and Baldwin A.S. Jr (2002). NF-κB as a therapeutic target in cancer. Trends Mol. Med. 8, 385-389.

Papa S., Zazzeroni F., Pham C.G., Bubici C. and Franzoso G. (2004a). Linking JNK signaling to NF-κB: a key to survival. J. Cell Sci. 117, 5197-5208.

Papa S., Zazzeroni. F., Bubici C., Jayawardena S., Alvarez K., Matsuda S., Nguyen D.U., Pham C.G., Nelsbach A.H., Melis T., De Smaele E., Tang W.J., D'Adamio L. and Franzoso G. (2004b). Gadd45ß mediates the NF-κB suppression of JNK signalling by targeting MKK7/JNKK2. Nat. Cell Biol. 6, 146-153.

Pham C.G., Bubici C., Zazzeroni F., Papa S., Alvarez K., Jayawardena S., De Smaele E., Cong R., Beaumont C., Torti F.M., Torti S.V. and Franzoso G. (2004). Ferritin heavy chain upregulation by NF-κB inhibits TNFα-induced apoptosis by suppressing reactive oxygen species. Cell 119, 529-542.

Rathmell J.C. and Thompson C.B. (2002). Pathways of apoptosis in lymphocyte development, homeostasis, and disease. Cell 109, S97-S107.

Reuther-Madrid J.Y, Kashatus D., Chen S., Li X., Westwick J., Davis R.J., Earp H.S., Wang C.Y. and Baldwin Jr A.S. Jr (2002). The p65/RelA subunit of NF-κB suppresses the sustained, antiapoptotic activity of Jun kinase induced by tumor necrosis factor. Mol. Cell Biol. 23, 8175-8183.

Rosenfeld M.E., Prichard L., Shiojiri N. and Fausto N. (2000). Prevention of hepatic apoptosis and embryonic lethality in RelA/TNFR-1 double knockout mice. Am. J. Pathol. 156, 997-1007.

Sabapathy K., Kallunki T., David J.P., Graef I., Karin M. and Wagner E.F. (2001). c-Jun NH2-terminal kinase (JNK)1 and JNK2 have similar and stage-dependent roles in regulating T cell apoptosis and proliferation. J. Exp. Med. 193, 317-328.

Sakon S., Xue X., Takekawa M., Sasazuki T., Okazaki T., Kojima Y., Piao J.H., Yagita H., Okumura K., Doi T. and Nakano H. (2003). NF-κB inhibits TNF-induced accumulation of ROS that mediate prolonged MAPK activation and necrotic cell death. EMBO J. 22, 3898-3909.

Salvesen G.S. and Duckett C.S. (2002). IAP proteins: blocking the road to death's door. Nat. Rev. Mol. Cell Biol. 3, 401-410.

Sanna M.G., da Silva Correia J., Ducrey O., Lee J., Nomoto K., Schrantz N., Deveraux O.L. and Ulevitch R.J. (2002). IAP suppression of apoptosis involves distinct mechanisms: the TAK1/JNK1 signaling cascade and caspase inhibition. Mol. Cell. Biol. 22, 1754-1766.

Schneider-Brachert W., Tchikov V., Neumeyer J., Jakob M., Winoto-Morbach S., Held-Feindt J., Heinrich M., Merkel O., Ehrenschwender M., Adam D., Mentlein R., Kabelitz D. and Schutze S. (2004). Compartmentalization of TNF receptor 1 signaling: internalized TNF receptosomes as death signaling vesicles. Immunity 21, 415-428.

Silverman N. and Maniatis T. (2001). NF-κB signaling pathways in mammalian and insect innate immunity. Genes Dev. 18, 2321-2342.

St. Clair D.K., Porntadavity S. and Kiningham K. (2002). Transcription regulation of human manganese superoxide dismutase gene. Methods Enzymol. 349, 306-312.

Stehlik C., de Martin R., Kumabashiri I., Schmid J.A., Binder B.R. and Lipp J. (1998). Nuclear factor (NF)-κB-regulated X-chromosome-linked iap gene expression protects endothelial cells from tumor necrosis factor alpha-induced apoptosis. J. Exp. Med. 188, 211-216.

Tak P.P. and Firestein G.S. (2001). NF-κB: a key role in inflammatory diseases. J. Clin. Invest. 107, 7-11.

Takekawa M. and Saito H. (1998). A family of stress-inducible GADD45-like proteins mediate activation of the stress-responsive MTK1/MEKK4 MAPKKK. Cell 95, 521-530.

Tang G., Minemoto Y., Dibling B., Purcell N.H., Li, Z., Karin M. and Lin A. (2001). Inhibition of JNK activation through NF-κB target genes. Nature 414, 313-317.

Tobiume K., Matsuzawa A., Takahashi T., Nishitoh H., Morita K., Takeda K., Minowa O., Miyazono K., Noda T. and Ichijo H. (2001). ASK1 is required for sustained activations of JNK/p38 MAP kinases and apoptosis. EMBO Rep. 2, 222-228.

Torti F.M. and Torti S.V. (2002). Regulation of ferritin genes and protein. Blood 99, 3505-3516.

Tournier C., Dong C., Turner T.K., Jones S.N., Flavell R.A. and Davis R.J. (2001). MKK7 is an essential component of the JNK signal transduction pathway activated by proinflammatory cytokines. Genes Dev. 15, 1419-1426.

Tournier C., Hess P., Yang D.D., Xu J., Turner T.K., Nimnual A., Bar-Sagi D., Jones S.N., Flavell R.A. and Davis R.J. (2000). Requirement of JNK for stress-induced activation of the cytochrome c-mediated death pathway. Science 288, 870-874.

Van Antwerp D.J., Martin S.J., Kafri T., Green D.R. and Verma I.M. (1996). Suppression of TNF-α-induced apoptosis by NF-κB. Science 274, 787-789.

Ventura J.J., Cogswell P., Flavell R.A., Baldwin Jr. A.S. and Davis R.J. (2004). JNK potentiates TNF-stimulated necrosis by increasing the production of cytotoxic reactive oxygen species. Genes Dev. 18, 2905-2915.

Wajant H., Pfizenmaier K. and Scheurich P. (2003). Tumor necrosis factor signaling. Cell Death Differ. 10, 45-65.

Wang C., Mayo M. and Baldwin Jr. A.S. (1996). TNF-α and cancer therapy-induced apoptosis: potentiation by inhibition of NF-κB. Science 274, 784-787.

Wertz I.E., O' Rourke K.M., Zhou H., Eby M., Aravind L., Seshagiri S., Wu P., Wiesmann C., Baker R., Boone D.L., Ma A., Koonin E.V. and Dixit V.M. (2004). De-ubiquitination and ubiquitin ligase domains of A20 downregulate NF-κB signalling. Nature 430, 694-699.

Zazzeroni F., Papa S., De Smaele E. and Franzoso G. (2003). Cell survival and Gadd45-factor deficiency. Nature 424, 742.

Zhang S.Q., Kovalenko A., Cantarella G. and Wallach D. (2000). Recruitment of the IKK signalosome to the p55 TNF receptor: RIP and A20 bind to NEMO (IKKγ) upon receptor stimulation. Immunity 12, 301-311.

Accepted July 15, 2005