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ROLE OF NF-KB IN THE CONTROL OF APOPTOTIC AND PROLIFERATIVE RESPONSES IN IL-2-RESPONSIVE T CELLS

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1. ABSTRACT

The NF-κB/Rel/IκB family of transcription factors regulates a number of genes involved in a wide variety of biological processes. The activation of p53, c-*myc* and Ras genes suggests a role for NF-κB in cell proliferation; NF-κB is also important in immune and inflammatory responses. By virtue of its role in apoptosis, NF-κB participates in the thymus as well as in embryonic development. The NF-κB family of transcription factors is also involved in viral transcription, transformation and in the development of some types of human cancers. Given the pivotal role of NF-κB, clarification is needed of the mechanisms through which its deregulation contributes to disease. Several aspects of NF-κB regulation, such as phosphatase involvement, the mechanism of IκB ubiquitination and the regulation of nuclear translocation, remain obscure. Here, we review and discuss the function of NF-κB activation in IL-2-stimulation and in apoptosis induced by IL-2 deprivation in T cells.

2. INTRODUCTION

Cells respond to intra- and extracellular signals by turning specific genes on or off and by modulating the extent of active gene transcription. Switching gene expression on and off is the responsibility of transcription factors, which operate singly or in association with other proteins. Transcription factors are usually organized in families, one of which is the NF-κB homo- or heterodimers. These proteins share the common property of being sequestered in the cytoplasm by the specific IκB proteins.

NF-κB was originally characterized as a lymphoid-specific protein that interacts with the immunoglobulin κ light chain gene enhancer sequences (1). Five mammalian NF-κB/Rel proteins have been cloned and characterized, including c-Rel, NF-κB1 (p50/p105), NF-κB2 (p52/p100), RelA/p65, and RelB (2-9) (Fig 1A). These proteins have common domains, such as the Rel homology domain, which functions in DNA binding, dimerization, interaction with IκB. This domain encompasses the nuclear localization signals (NLS). NF-κB1 and NF-κB2 contain multiple copies of the ankyrin repeat at their C-termini. Processing of the precursors p105 and p100, which is signal-dependent, leads to the production of the p50 and p52 subunits. The cytoplasmic precursors p105 and p100 perform inhibitory functions by preventing their respective processed products binding to κB sites (10-13). NF-κB processing requires ATP and is mediated by a ubiquitin-dependent proteasome degradation pathway (14). Other members of the NF-κB/Rel protein family are not generated from precursor proteins and have a carboxy-terminal transactivation domain (15). The κB sites are present in the regulatory regions of genes involved in immune (IL-2, IL-2Rα) and inflammatory responses (IL-1, IL-6, TNFα, TNFβ), as well as in genes of viruses, NF-κB/Rel members, IκB members, growth control proteins (p53, c-*myc*, Ras) and adhesion molecules (15-19). NF-κB/Rel protein functions are involved in cell transformation, tumor growth, apoptosis, embryonic liver development and functional differentiation of immune cells (20-23).

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In most cell types, NF-κB is associated with the inhibitor IκB in the cytoplasm. Following activation, a small fraction of NF-κB dissociates from the inhibitor and is translocated to the nucleus (24). The mechanism of NF-κB nuclear translocation following IκB degradation is speculated to include phosphorylation or dephosphorylation events that target IκB for ubiquitination and subsequent proteasome-dependent degradation (25, 26). There are at least nine mammalian IκB molecules with distinct and overlapping inhibitory specificities, including IκBα, IκBβ, IκBγ, IκBε, Bcl-3, p105, p100, A238L and IκBR (12, 13, 27-31) (Fig 1A). The IκB family proteins share conserved motifs referred to as ankyrin repeats. These motifs are required for association of IκB proteins with NF-κB/Rel proteins (15, 29). In addition, the IκB proteins contain a acidic carboxy-terminus region (30) and a carboxy-terminus domain containing a Pro, Glu/Asp, Ser and Thr-rich PEST sequence, which are implicated in regulating protein half-life (32). Deletion of this sequence partially protects IκBα from degradation (33, 34). Phosphorylation of IκB is observed in many cell types following stimulation (35, 36). The kinase or kinases that phosphorylate IκBα upon stimulation recognize the same serine-32 and -36 residues. Recent studies demonstrate that the basal phosphorylation of IκBα occurs at the carboxy-terminal casein kinase II sites in the PEST region (37).

Prior to NF-κB activation, IκB undergoes complete degradation following stimulation, (12, 35, 38). IκB degradation is an efficient process that can be inhibited by serine protease inhibitors, suggesting that it is an obligatory step in NF-κB activation (30). Thus, the sequence of events leading to NF-κB activation may require phosphorylation of IκBα at serine residues, followed by phosphorylation-dependent multi-ubiquitination at lysine residues, degradation of IκBα by a ubiquitin-dependent proteasome and, finally, the release of free NF-κB transcription factor (Fig 1B).

Some kinases have been implicated in NF-κB activation. The best characterized kinase is probably the double-stranded RNA-activated kinase (PKR), which phosphorylates IκBα *in vitro* (39). Raf-1 has also been proposed to target IκB (40, 41), and experiments using dominant negative mutants have implicated ζPKC as a regulator of NF-κB activation (42). Finally, εPKC has also been suggested to induce NF-κB activation (42, 43). Several inducers trigger NF-κB activation, including TNF, IL-1, lipopolysaccharide (LPS), phorbol esters, okadaic acid, serum growth factors and nitric oxid (NO) (2, 3, 5, 15, 44, 45).

Phosphatases probably play an important role in NF-κB activation, either by regulating the kinase pathways or by direct dephosphorylation of IκB. Based on inhibition by cyclosporin A and transfection studies, the phosphatase calcineurin appears to be involved in NF-κB activation in T cells (46). Calcineurin increases NF-κB activity by increasing IκB phosphorylation and degradation, leading to an increase in the level of active nuclear NF-κB (47). Calcineurin is also involved in p105/NF-κB1 induction and in the decrease in p50 dimer levels in the nuclei of activated T cells (48). The result of these calcineurin-mediated processes would be to increase the ratio of active to inactive complexes, and thus to potentiate transactivation from the NF-κB site (48, 49). The serine/threonine phosphatase inhibitors PP1 and PP2A also activate NF-κB, suggesting the implication of a phosphatase in regulating aspects of the pathway.

Once in the nucleus, the NF-κB proteins not only bind to DNA, but also interact with many other proteins that may also participate in NF-κB regulation. c-Rel binds directly to the TATA binding protein and TFIIB (50, 51), and to the RNA polymerase II subunits. In some cases, a high-mobility-group protein [HMG-I(Y)] is required as coactivator for transcriptional activation (52). There are also activator proteins, such as the helix-loop-helix (HLH) family proteins or steroid receptors, which can interact with the NF-κB proteins to activate, repress, enhance or decrease transcription of various genes (15). Thus, NF-κB proteins, once in the nucleus, are subject to additional levels of control.

3. NF-κB CONTROLS IL-2 GENE EXPRESSION

IL-2, a 15 kDa glycoprotein, is produced by some T cells; it acts on T cells as a major growth-promoting factor. IL-2 promoter contains an enhancer sequence located between nucleotides -548 to +39 relative to the transcription initiation site. Nuclear factor of activated T cells (NFAT), Oct-1, activating protein-1 (AP1) and NF-κB are transcription factors that bind to identified positive elements in the IL-2 promoter. Mutations in the NF-κB site of the IL-2 promoter are less deleterious than mutations in the binding sites for other transcription factors. Although most NF-κB/Rel family proteins are present in T cells, the heterodimer p50/p65 (NF-κB1/Rel A) is the major nuclear factor binding to the NF-κB site of the IL-2 promoter (53, 25, 26). In response to extracellular stimuli, p50/p65 is released in T cells from an inactive cytoplasmic pool by rapid phosphorylation and subsequent degradation of the inhibitor IκB (33). This release unmasks the nuclear localization signal of NF-κB and leads to its translocation to the nucleus (3). Finally, phosphorylation of NF-κB may be required for its fully functional activity (48, 49, 25, 26).

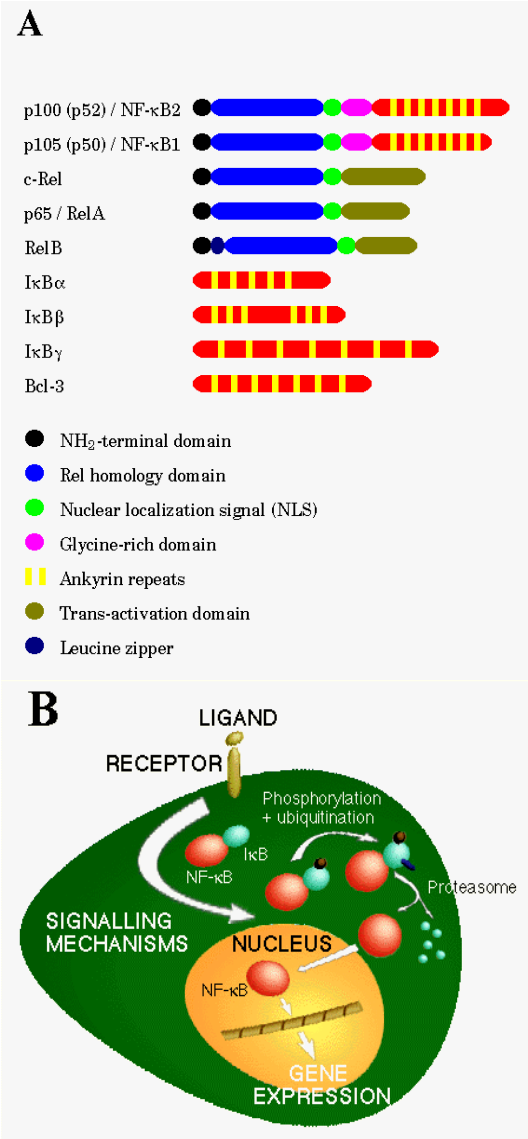

Figure 1.

A. Schematic representation of some members of the Rel/NF-κB and IκB families.

B. NF-κB regulation. In response to external signals, phosphorylated IκB binds to NF-κB factor. This phosphorylation targets IκB for ubiquitination. NF-κB bound to ubiquitinated and phosphorylated IκB is cleaved by a proteasome complex. Free NF-κB translocates to the nucleus and induces expression of certain genes.

c-Rel is also a significant component of the complexes binding to the NF-κB site of the IL-2 promoter, and its function may be related to the maintenance rather than to repression of late transcription of IL-2. p50 dimers repress IL-2 gene transcription (49). The function of p52 in IL-2 transcription has not been studied.

4. NF-κB AND THE IL-2 RECEPTOR

The IL-2 receptor is composed of at least three distinct subunits, p55, p70 and p64, or α, β and γ chains, respectively; this trimolecular complex binds IL-2 with a high affinity (54-56). Expression of the p55 subunit is inducible in T cells by activation through the T cell receptor (57). The cytoplasmic region of p55 subunit has 13 amino acids, including serine and threonine residues as potential phosphorylation targets. Deletion of this region does not affect the capacity of the high affinity IL-2 receptor to transmit IL-2-mediated proliferative signals.

Previous studies demonstrated that inducible IL-2Rα expression is at least partially regulated by a potent enhancer located between nucleotide positions -299 and -228 relative to the major transcription initiation site (58). This enhancer is termed the positive regulatory region I (PRRI) and contains NF-κB, serum response factor (SRF), SP1 and UE-1 motifs (58-62). These binding sites are important in IL-2Rα gene activation in response to several stimuli, including the transactivator protein, Tax, of the human T cell lymphotropic virus type I, PMA, TNFα, IL-2 and IL-1 (19, 60, 62-67).

Internal deletions within the IL-2Rα promoter suggested the presence of other positive regulatory elements located between nucleotides -137 and -64, termed positive regulatory region II (PRRII). This region contains sites for at least two DNA-binding proteins, Elf-1 (68), a member of the Ets family, and the nonhistone chromatin-associated proteins, HMG-I(Y) (69-71). Deletion of the binding sites for these proteins profoundly reduced IL-2Rα gene transcription, even in the presence of an intact upstream enhancer (PRRI). Elf-1 specifically binds to p50 and c-Rel *in vitro*, suggesting that these protein-protein interactions may mediate the transcriptional coordination between PRRI and PRRII (72) (Fig. 2).

Elf-1 is the first Ets family protein known to interact with NF-κB family members (73). Mapping of the Elf-1 interaction domain with c-Rel revealed that the Ets domain is necessary and sufficient to mediate this interaction (74). Elf-1 interaction with p50 is enhanced by the presence of HMG-I(Y), which has also been shown to associate with p50 (52, 75). Elf-1 may be involved in the selective binding and stabilization of specific NF-κB family proteins to PRRI during T cell activation. Elf-1 can interact with p50, but not with its precursor p105, suggesting that the interaction is masked in p105.

c-Rel/ p50 heterodimers also bind to the PRRI enhancer of the IL-2Rα gene and the amount of heterodimer present correlates with the level of

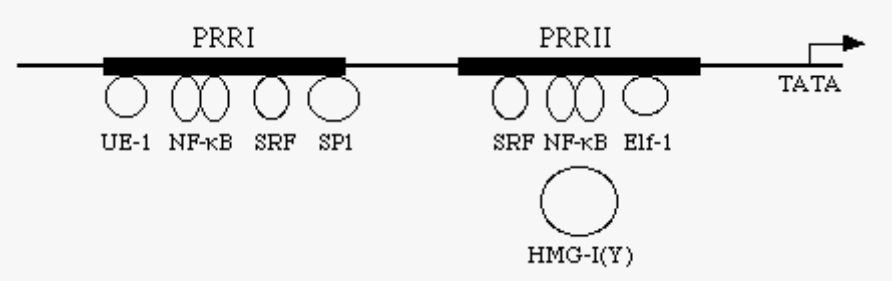

Figure 2. Schematic diagram of the IL-2 receptor, a 5' regulatory region, including positive regulatory regions PRRI and II.

IL-2Rα gene expression. c-Rel or p65 can cooperate with SRF in IL-2Rα promoter activation (76). Synergy of c-Rel and SRF may reflect interaction of these proteins with basal transcription factors. Finally, c-Rel can also associate with TATA binding proteins of the transcription factor IID complex to mediate transcription activation of IL-2Rα gene expression (50, 77).

The IL-2Rα gene is not only expressed in mature activated T cells, but is also found early in T cell ontogeny, before expression of TCR genes (78). It has been proposed that this early expression could involve the NF-κB/SRF interaction (79). The recent observation that NF-κB proteins can be found in thymocytes (80, 81) suggests that NF-κB/SRF interactions may also be important in early T cell development.

5. NF-κB AND APOPTOSIS

Although past evidence linked the NF-κB family of transcription factors to the control of apoptotic responses, these relationships have been addressed only recently. Initial studies assigned c-Rel a role in apoptosis induction, since elevated subunit expression levels of these molecules were associated with programmed cell death both in the developing avian embryo and in bone marrow cells *in vitro* (82). Tetracycline-dependent induction of c-Rel expression in stably transfected HeLa cells causes the onset of apoptosis (31, 83). In this case, apoptosis was associated with cell cycle arrest at G_1/S, inhibition of E2F DNA binding activity, accumulation of hypophosphorylated Rb, inhibition of Cdk2 kinase activity and an increase in $p21^{Cip1/Waf1}$ transcript levels. Additional data support the assignment of apoptotic-inducing properties to NF-κB complexes; thus, radiation-induced apoptosis of fibroblasts from ataxia-telangiectasia (AT) patients, which exhibit a constitutive NF-κB-like activity, was reduced by a dominant negative IκBα mutant (84). The pro-apoptotic capability of the c-Rel protein is not shared by its viral counterpart, since both the use of a temperature-sensitive v-Rel mutant (85) and downregulation of v-Rel expression in chicken spleen cells through a tetracycline-controlled system suggest a role for v-Rel in apoptosis suppression (31, 85).

In contrast to the results obtained with c-Rel, p65/RelA acts as a potent apoptosis inhibitor. The first evidence came from the study of RelA knockout mice, which suffer from massive hepatic apoptotic death and die during embryonic life (21). These observations led to a hypothesis in which RelA drives mechanisms protecting fetal hepatocytes from apoptotic signals delivered by resident hematopoietic cells. The RelA knockout phenotype closely resembles that of c-*jun*-deficient mice, suggesting similar functions for RelA-containing NF-κB dimers and c-*jun* in fetal liver development.

Further evidence supports the role of RelA in apoptosis suppression. Thus, the presence of RelA has been correlated with resistance to TNF-α-induced apoptosis in mouse fibroblasts and macrophages (86). RelA function in this context has been associated with the induction of anti-apoptotic genes that result from the interaction of TNF-α with its type I receptor. As occurs with TNF-α, other apoptotic stimuli that induced NF-κB nuclear translocation such as ionizing radiation and the chemotherapeutic compound, daunorubicin, did not kill cells when NF-κB function was allowed (87). Stable transfection of primary mouse embryo fibroblasts or Jurkat human lymphoma cells with a dominant negative IκBα mutant that is defective in phosphorylation and thus not susceptible to degradation, rendered these cell types susceptible to TNF-α-induced apoptosis (88). Transgenic mice expressing a dominant negative IκB mutant under the control of the T cell-specific *lck* promoter also showed a loss of $CD8^+$ T cells in the thymus and enhanced sensitivity to activation-induced cell death (31).

6. A DUAL ROLE FOR NF-κB GENE FAMILY MEMBERS IN PROGRAMMED CELL DEATH

A functional distinction has been suggested with regard to the involvement of different NF-κB monomers in apoptosis regulation; c-Rel promotes cell death, whereas RelA protects from it. These dual responses do not derive simply from the fact that

these proteins are potential dimerization partners of a single complex. It has been proposed that a difference in the κB-specific motifs exists by which anti-apoptotic genes bind RelA complexes selectively, whereas c-Rel-containing dimers specifically bind to pro-apoptotic genes. Alternatively, c-Rel overexpression could prevent formation of RelA dimers required for the activation of anti-apoptotic genes. Even if any of these hypotheses is proven correct, none can account for the recent data that rule out such a simple scheme. First, apoptosis-promoting activity has also been reported for RelA. Thus, serum deprivation-induced apoptosis of 293 cells is characterized by an increase in RelA-containing NF-κB activity (89). Both effects, cell death and NF-κB activation, may be prevented by Bcl-2 or a dominant negative RelA mutant. c-Rel prevents apoptosis induced by either IgM crosslinking or a protease inhibitor in the WEHI 231 immature B cell lymphoma line, since microinjection of anti-c-Rel antibodies or an IκBα-GST fusion protein promotes cell death (90). Studies of normal and transformed murine B cells suggest that reduction in NF-κB DNA binding activity as a consequence of surface IgM ligation may be a determining event for the onset of apoptosis.

The pro- or anti-apoptotic properties of different NF-κB subunits is far from clear, and it is possible that a dual regulation may be exerted by the same proteins. Thus, studies of cellular response control by signaling mediators have led to the identification of molecules with multifunctional capabilities. Examples include proteins that act as cell cycle and cell death regulators, such as the tumor suppressor p53, the E2F transcription factors, the protooncogenes *bcl*-2 and c-*myc* (91-95). Other examples include the small G proteins of the Ras superfamily, of which both Rho-like and Ras-like proteins have been linked to cell proliferation (96-100) and to a dual control of apoptosis, either as suppressors or promoters of cell death (100-103; Gómez *et al*., submitted). Apoptosis is now envisioned as one of the options to be selected by the cell during cell cycle progression, and the switching from proliferation to cell death may be determined by a defective proliferative signal. If NF-κB activity is functionally linked to such mediators, it would not be surprising that this family of transcription factors would also be involved in driving diverse or even opposite cellular responses. There is evidence linking NF-κB activity with the functions of mediators implicated in cell proliferation. NF-κB activates p53, c-*myc* and c-H-ras genes. In addition, Ras has been proposed as the initiator of a signaling pathway that induces NF-κB through the sequential activation of the atypical protein kinase C (PKC) ζ isoform and a putative IκB kinase (43). Other reports have localized NF-κB in a pathway led by the Rho family proteins RhoA, Rac1 and Cdc42, that results in NF-κB activation through IκBα depletion. The same set of experiments showed involvement of RhoA and Cdc42, but not Rac1, in TNF-α-induced NF-κB activity (83). Relationships between NF-κB and several molecules involved in apoptosis control have been demonstrated. Furthermore, NF-κB activity increases following certain types of stimulation often associated with the onset of cell death in several systems, such as TNF-α, ultraviolet light, H_2O_2, calcium ionophores, phorbol esters or ceramides. Although in the case of TNF-α, as mentioned above, the apoptotic and NF-κB activation pathways seem to be divergent, the possible relevance of NF-κB in the cell death signals putatively triggered by other stimuli remains to be clarified. Finally, Fas receptor stimulation is followed by NF-κB DNA binding activity in some, but not all, cell types. In a cell line in which Fas triggers NF-κB activation, no apoptosis was detected when Fas ligation was accompanied by inhibition of NF-κB (88), suggesting that, in contrast to TNF-α-induced signaling, Fas-mediated cell death does not rely on NF-κB activity.

A few clinical implications of NF-κB function in apoptosis regulation are worth mentioning. TNF-α has been used as a therapeutic agent to trigger the killing of tumor and infected cells. However, the early expectations were not fulfilled, and transformed cells are often resistant to TNF-α-induced apoptosis. In the case of viral infection, this may be partially explained by the fact that some viruses express gene products promoting cellular NF-κB activity, making infected cells resistant to TNF-α-induced apoptosis. Moreover, NF-κB activation may also result in proviral transactivation, as is the case of HIV (104). There again, the dual role of NF-κB might cause the double effect of promoting both viral expression and the survival of infected cells. The pro-apoptotic effect of TNF-α, useful for the therapeutic removal of infected or tumorigenic cells, may therefore be productively enhanced by inhibition of NF-κB, either through the administration of suppressor drugs such as glucocorticoids, antioxidants (105) or Cu^{2+} (106), or through the genetic delivery of IκB proteins.

7. ROLE OF NF-κB IN IL-2-TRIGGERED T CELL RESPONSES

NF-κB activation following ligation of the IL-2 receptor is an event involved in the signal transduction pathways triggered by this lymphokine. It remains to be defined, however, which cellular responses are controlled by NF-κB-dependent gene transactivation and which signals are involved in activation and nuclear translocation of NF-κB dimers.

The IL-2 receptor signaling system has been explained as a three-channel model, in which at least three different pathways mediate the flow of mitogenic and survival-promoting signals (107, 108). According to this scheme, one of the pathways

(channel 1) proceeds through protein tyrosine kinase activity, Ras and the MAPK cascade, leading to expression of the protooncogenes c-*fos* and c-*jun*. Channel 2 is proposed to be initiated by the protein tyrosine kinase Syk and to be responsible for c-*myc* gene induction. Finally, channel 3 results in *bcl*-2 expression, and progression through a Rho-, PI3 kinase- and ζPKC-mediated signaling pathway (96; Gómez *et al.*, submitted). This last pathway is also involved in IL-2-promoted regulation of actin cytoskeleton organization. In the murine TS1αβ T cell line, cooperation among the three channels triggers cell proliferation, while cooperation between only two, when one of them is channel 1, maintains cell survival with no mitogenic effect (108).

IL-2 induction of nuclear NF-κB dimers with DNA binding activity is sensitive to the immunosuppressant, rapamycin, both in the TS1αβ and TS1β cell lines (109). The cellular effects of this drug have been attributed to a specific inhibition of the 70 kDa kinase of the S6 ribosomal protein, $p70^{s6k}$ (110-112). However, rapamycin action also appears to affect PI kinase activities (113-114) and formation of active cyclin-cdk complexes (115). In TS1αβ cells, rapamycin inhibits IL-2-induced PI3 kinase activity and cell proliferation. Rapamycin also modifies the electrophoretic mobility shift pattern of NF-κB induction by IL-2 (109), suggesting that the pathway responsible for NF-κB activation and nuclear translocation by IL-2 in TS1αβ cells is channel 3. Recent evidence indicates that IL-2 stimulation of NF-κB in the same cell line is inhibited to a similar extent by *Clostridium difficile* toxin B, a specific Rho protein inhibitor, or by wortmannin, a compound that produces covalent inactivation of PI3 kinase (Gómez *et al.*, unpublished results). The results suggest that NF-κB activation by IL-2 might occur through a Rho-PI3 kinase-ζPKC pathway that triggers the activation of an IκB kinase and subsequent release of free active NF-κB complexes. These data support earlier studies that proposed the participation of ζPKC as a putative inducer of NF-κB activation (43). Rho family proteins have also shown implication in the activation of NF-κB complexes (83).

One possibility that may be inferred from the above data is that NF-κB might be involved in *bcl*-2 gene induction. In fact, NF-κB consensus binding sequences exist within the *bcl*-2 gene promoter, and our recent evidence indicates that a correlation exists between the IL-2-induced appearance of nuclear active NF-κB complexes and *bcl*-2 expression, whereas none of these events occurs when cells are stimulated with IL-4 (Gómez *et al.*, unpublished results). If these hypotheses prove correct, it might be deduced that NF-κB would control both mitogenic and survival signals in IL-2 receptor signaling, as occurs with Rho. Inhibition of Rho activity prevents IL-2-induced proliferation but does not affect cell survival, concurring with the three-channel model applied to the TS1αβ cell line. Conversely, a constitutively active form of Rho protects TS1αβ cells from lymphokine withdrawal-induced apoptosis. This latter effect is due to the fact that Ras is active in lymphokine-deprived TS1αβ cells Rho activation thus complements the Ras signal, providing a rescue pathway that abolishes programmed cell death (Gómez *et al.*, submitted). In summary, NF-κB may act as a regulatory step for stimulation of both cell survival and proliferation by IL-2 in T cells. Whether the complete Rho-delivered signal proceeds through NF-κB activation or branches at an earlier step will determine whether NF-κB control over cellular responses is comparable to that exerted by Rho proteins.

8. CONCLUDING REMARKS

We have addressed the involvement of various NF-κB family members in the activation of the promoters of the genes encoding two immunologically relevant molecules, the cytokine IL-2 and the IL-2Rα chain. We have also compiled and discussed the available evidence that links NF-κB complexes to the control of apoptotic responses, either as suppressors or inducers of cell death. Finally, we have reviewed the involvement of NF-κB proteins in IL-2 receptor signaling.

Although preserving a prominent role as a critical mediator of cellular immune regulation, the latest findings on the biology and biochemistry of NF-κB highlight multifunctional implications for these transcription factors in cell responses and development. NF-κB appears to play a dual role in apoptotic response regulation, depending on the relationship between NF-κB activity and key signaling molecules in cellular responses. It is expected that, as for other multifunctional mediators, future studies of NF-κB activity will analyze the simultaneous delivery of different signals and how they interact and complement each other at the level of gene activation and repression to elicit global responses. According to current evidence, NF-κB activity may be regarded as a step in signal integration. For example, at least two different signaling pathways, mediated by calcium and PKC/Ras/Raf, respectively, may act synergistically in T cells to induce NF-κB activation (83) . This synergy may reflect the need for two different kinases acting simultaneously on IκB. Phosphorylation of serines 32 and 36 of IκBα is required for its degradation and subsequent NF-κB nuclear translocation. Serine 32, but not serine 36, is a target for the mitogen-activated kinase, pp90rsk, which acts downstream of MAPK, MEKK-1 and MEKK-3 within a putative PKC/Ras/Raf pathway and is activated in response to PMA, LPS or okadaic acid. In addition, a constitutive Raf kinase can only activate NF-κB in combination

with a constitutive form of calcineurin. These data suggest that IκBα receives signals from two convergent signaling pathways, mediated by Ras and Ca^{2+}, respectively, that result in NF-κB activation (116).

Hence, research on the onset of gene expression by NF-κB transcription factors and on their functional involvement in the control of cellular responses will help to elucidate the molecular control of the cell fate.

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