



Diverse agents act at multiple levels to inhibit the Rel/NF- κ B signal transduction pathway

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Rel/NF- κ B transcription factors regulate several important physiological processes, including developmental processes, inflammation and immune responses, cell growth, cancer, apoptosis, and the expression of certain viral genes. Therefore, they have also been sought-after molecular targets for pharmacological intervention. As details of the Rel/NF- κ B signal transduction pathway are revealed, it is clear that modulators of this pathway can act at several levels. Inhibitors of the Rel/NF- κ B pathway include a variety of natural and designed molecules, including anti-oxidants, proteasome inhibitors, peptides, small molecules, and dominant-negative or constitutively active polypeptides in the pathway. Several of these molecules act as general inhibitors of Rel/NF- κ B induction, whereas others inhibit specific pathways of induction. Inhibitors of Rel/NF- κ B are likely to gain stature as treatments for certain cancers and neurodegenerative and inflammatory diseases.

Keywords: Rel; NF- κ B; I κ B; signal transduction; NF- κ B inhibition; IKK

Introduction

As this issue attests, the Rel/NF- κ B transcription factors comprise one of the most highly-studied groups of eukaryotic gene regulatory proteins. They control the expression of genes involved in many critical physiological responses, including immune and acute phase inflammatory responses, cell adhesion, differentiation, redox metabolism, and apoptosis (reviewed in Ghosh *et al.*, 1998; Pahl, 1999). Vertebrate Rel/NF- κ B transcription complexes can be any of a variety of homo- and heterodimers formed by the subunits p50, p52, RelA (p65), RelB and c-Rel. These complexes bind to DNA regulatory sites, called κ B sites, to (generally) activate specific target gene expression. The target gene specificity is thought to arise primarily from the specific Rel/NF- κ B complexes that are in different cell types and the distinct κ B target site binding specificities of different Rel/NF- κ B complexes.

In most cell types, NF- κ B (the name for p50-RelA, the most common heterodimer) is retained in the cytoplasm in an inactive form through association with any of several I κ B inhibitor proteins (I κ B α , - β , - ϵ , - γ , p105, p100 and Bcl-3; for review, see Whiteside and

Israël, 1997). In response to a wide array of stimuli, many of which are involved in intercellular communication, such as proinflammatory molecules (Pahl, 1999), I κ B is rapidly phosphorylated, ubiquitinated, and degraded. The liberated transcription complex then translocates to the nucleus where it can modulate specific gene expression.

The phosphorylation and the degradation of I κ B α have received great attention, as key steps for the regulation of Rel/NF- κ B complexes (see Karin, 1999, this issue). The I κ B α kinase (IKK) activity resides in a high molecular weight complex containing at least two kinase subunits, IKK α and IKK β , and an associated modulator (called IKK γ , NEMO, or IKKAP). Under certain conditions, a fourth molecule, called IKAP, has also been found associated with the IKK complex, but its function is not known. The IKKs share much structural similarity and form homo- and heterodimers. After stimulation of cells by agents such as tumor necrosis factor α (TNF α) or Interleukin-1 (IL-1), the IKK complex is activated by upstream kinases through phosphorylation of specific serine residues in the activation domain of each IKK subunit. Among the possible upstream kinases, NIK, MEKK-1, RIP, and IRAK have been found to act differently according to the incoming stimulus (see Karin, 1999, this issue). The activated IKK complex can then phosphorylate I κ B α on two serine residues (Ser 32 and 36 in human I κ B α). Phosphorylation of I κ B α by IKK signals it for ubiquitination at specific N-terminal lysine residues by the HOS-SCF E3 ubiquitin ligase complex (Fuchs *et al.*, 1999), which targets I κ B α for degradation by the 26S proteasome. NF- κ B is now free to enter the nucleus. However, there are alternative pathways for activation of NF- κ B: for example, ultraviolet irradiation NF- κ B activation does not appear to use the IKK complex, and phosphorylation of I κ B α at a tyrosine residue leading to activation of NF- κ B has also been reported (Imbert *et al.*, 1996; Li and Karin, 1998).

Therefore, several steps leading to the activation of an NF- κ B target gene are regulated by protein complexes that act as homo- and heterodimers and/or by families of closely related, interacting proteins. This adds additional levels of complexity to the regulation of NF- κ B activity. For example, as described above, IKK α and IKK β are active as dimers. However, they appear to have distinct physiological functions in mice in that disruption of each IKK gene leads to quite different phenotypes (Karin, 1999, this issue). Furthermore, IKK α and IKK β can be activated independently by different upstream signals. Moreover, the IKKs are likely to phosphorylate substrates in addition to the I κ Bs. Similarly, the different I κ B proteins have different affinities for the diverse Rel/NF- κ B dimers.

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Thus, different I κ B proteins (which may themselves be preferred targets of different IKK complexes) can indirectly regulate the expression of distinct sets of genes, which are targets of specific Rel/NF- κ B complexes. Finally, Rel/NF- κ B is a family of dimeric transcription factors, which have preferred target κ B sites and, thus, have distinct sets of target genes. Therefore, the activities of all interacting proteins in the Rel/NF- κ B signaling pathway are no doubt dependent on the precise nature and intensity of the upstream activating signals. These interacting levels must be considered when one wants to inhibit a given NF- κ B response. Thus, there are broad-range inhibitors with a wide range of action, that act at an early step of induction. In addition, there are more specific inhibitors, often designed according to details of the Rel/NF- κ B molecular signaling pathway (Figure 1).

Because of the multitude of cellular and organismal processes affected by Rel/NF- κ B signaling, there has been great interest in modulators of this pathway. For example, because Rel/NF- κ B complexes are known to play key roles in the inflammatory response and in the inhibition of apoptosis (Barkett and Gilmore, 1999, this issue; Ghosh *et al.*, 1998; Pahl, 1999, this issue), specific and efficient inhibitors of Rel/NF- κ B have been sought. Due to the large number of inducers of Rel/NF- κ B (Pahl, 1999, this issue) and the various levels of regulation of this pathway (Karin, 1999, this issue),

inhibitors also include a large variety of molecules that act at multiple levels.

The cascade of Rel/NF- κ B signaling events provides several key steps for specific inhibition of NF- κ B activity. That is, inhibition of Rel/NF- κ B activation can occur by: (1) blocking the incoming stimulating signal at an early stage and thus blocking its general effect; (2) interfering with any step in the NF- κ B activation pathway by blocking a specific member of the cascade; or (3) blocking NF- κ B nuclear activity, that is, inhibiting its translocation to the nucleus, its binding to DNA or its interactions with the basal transcription machinery. Of course, each of these three major steps would not be susceptible to the same types of inhibitors. This review will attempt to classify the multitude of NF- κ B inhibitors that have been reported and to describe their mechanisms of action.

Anti-oxidant molecules

In many, but not all cell types, NF- κ B can be activated by oxidants and its induced activity can be inhibited by anti-oxidants (Li and Karin, 1999; Pahl, 1999, this issue). However, neither the target for oxidant-induced activation nor the exact pathway used by such molecules to activate NF- κ B are known. The recent discovery of numerous participants in the activation cascade triggered by TNF α (TRAFs, NIK, IKKs) may enable the identification of the redox-responsive target(s). It has also been reported that various agents like TNF α , IL-1, phorbol ester (e.g., PMA), lipopoly-saccharide (LPS) or ultraviolet (UV) light can induce oxidative stress as well as induce NF- κ B (Siebenlist *et al.*, 1994). This general finding suggested that agents with anti-oxidant activity would also inhibit activation of NF- κ B as induced by a variety of agents.

Consistent with this, many compounds such as thiol anti-oxidants (e.g., NAC, PDTC) and calcium chelators (e.g., EGTA, lacidipine) have been used to inhibit hydrogen peroxide-induced NF- κ B activation. Presumably, many of these agents act by scavenging reactive oxygen intermediates (ROIs) (Sen *et al.*, 1996b). In addition, inhibitors of mitochondrial electron transport that suppress ROI production (like rotenone) or overexpression of enzymes that are involved in regulation of the redox state of the cell can block TNF α -induced activation of NF- κ B (Manna *et al.*, 1998, 1999b; Schulze-Osthoff *et al.*, 1993). In most cases, the inhibitory effects of anti-oxidants on NF- κ B have been shown by demonstrating reduced κ B site DNA-binding activity after stimulation by a given agent, without determining the precise step in NF- κ B activation that is blocked. For certain anti-oxidants (glutathione for example), NF- κ B activation appears to be inhibited at an early step, in that I κ B α phosphorylation and degradation are blocked (Cho *et al.*, 1998). However, a recent report indicated that the anti-oxidant NAC blocks TNF α -induced degradation of I κ B α but does not affect its phosphorylation or induced IKK activity (Li and Karin, 1999).

Therefore, all anti-oxidants may not act at the same level to inhibit NF- κ B. Caffeic acid phenethyl ester (CAPE) is a phenolic anti-oxidant, a structural relative of flavonoids, which are the active components of propolis from honeybee hives. CAPE has been

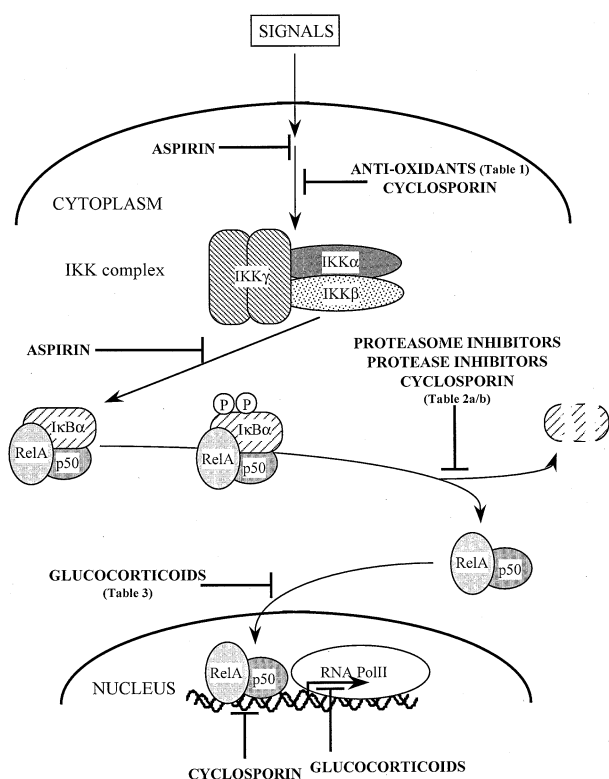


Figure 1 Level of action of various inhibitors of the Rel/NF- κ B signal transduction pathway. Various extracellular signals can initiate a pathway that activates the I κ B kinase complex (IKK), leading to the phosphorylation, ubiquitination and degradation of I κ B, which allows NF- κ B to enter the nucleus and regulate specific gene expression. Some of the inhibitors that block this pathway are shown in bold. See also the text and the indicated Tables for more details and other inhibitors that act at these specific points

reported to have anti-tumor activity in rat and human melanoma and breast carcinoma cell lines (Grunberger *et al.*, 1988). CAPE can also block the ability of TNF α , phorbol esters, ceramide, okadaic acid, and hydrogen peroxide to activate NF- κ B. Unlike other anti-oxidants, CAPE does not affect I κ B α phosphorylation and degradation (Natarajan *et al.*, 1996). Rather, CAPE-mediated inhibition of NF- κ B may involve direct and specific interference of DNA binding by NF- κ B, and this effect on DNA binding can be reversed by reducing agents like dithiothreitol and β -mercaptoethanol (Natarajan *et al.*, 1996).

Table 1 lists the many anti-oxidant molecules that have been shown to inhibit NF- κ B. It is important to remember that these molecules may function in at least two ways to block activation of NF- κ B. On one hand, anti-oxidants could block the production of ROIs that lead to degradation of I κ B α . On the other, DNA binding by NF- κ B could be directly regulated by the redox potential of the cell; for example, at least *in vitro*, the oxidation state of NF- κ B seems important for its interaction with DNA (Yang *et al.*, 1995), and its DNA binding can be blocked by interaction with thiol reactive metals (Shumilla *et al.*, 1998). Animal models have indicated that the anti-oxidant PDTC may have clinical applications for the treatment of septic shock

and certain retinal neovascular diseases, due to the ability of PDTC to block NF- κ B induction (Lauzurica *et al.*, 1999; Yoshida *et al.*, 1999).

Proteasome inhibitors

The last common step before NF- κ B is freed from the cytoplasm is the degradation of ubiquitinated I κ B by the 26S proteasome (Karin, 1999, this issue; Palombella *et al.*, 1994). Thus, inhibitors of the ubiquitin-proteasome pathway suppress activation of NF- κ B by stabilizing I κ B. Proteasome inhibitors and protease inhibitors that block NF- κ B activity are listed in Table 2a. The inhibitors described below can all penetrate cells and inhibit NF- κ B activation in a dose-dependent manner by blocking proteasome-mediated degradation of I κ B (but not its phosphorylation).

The best-characterized proteasome inhibitors are peptide aldehydes. These molecules inhibit the chymotrypsin-like activity of the proteasome complex (one of the five protease activities of the eukaryotic proteasome), but with distinct efficiencies. ALLnL, also called calpain inhibitor I or MG101, is a cysteine protease inhibitor, but is a less potent inhibitor of the proteasome than MG132 and MG115 (Grisham *et al.*, 1999; Jobin *et al.*, 1998a; Palombella *et al.*, 1994).

Table 1 Anti-oxidants that have been shown to inhibit activation of NF- κ B

<i>Molecule</i>	<i>References</i>
α -lipoic acid	Sen <i>et al.</i> , 1998; Suzuki <i>et al.</i> , 1992
α -tocopherol	Islam <i>et al.</i> , 1998
Anetholdithiolthione (ADT)	Sen <i>et al.</i> , 1996a
Butylated hydroxyanisole (BHA)	Israël <i>et al.</i> , 1992; Schulze Osthoff <i>et al.</i> , 1993
Cepharanthine	Okamoto <i>et al.</i> , 1994
Caffeic Acid Phenethyl Ester (3,4-dihydroxycinnamic acid, CAPE)	Natarajan <i>et al.</i> , 1996
Catechol derivatives	Suzuki and Packer, 1994
Diethyldithiocarbamate (DDC)	Schreck <i>et al.</i> , 1992b
Diferoxamine	Sappey <i>et al.</i> , 1995; Schreck <i>et al.</i> , 1992b
Dihydrolipoic acid	Suzuki <i>et al.</i> , 1992, 1995
Disulfiram	Schreck <i>et al.</i> , 1992b
Dimethyldithiocarbamates (DMDTC)	Pyatt <i>et al.</i> , 1998a
Curcumin (Diferuloylmethane)	Singh and Aggarwal, 1995b
Ebselen	Schreck <i>et al.</i> , 1992b
EPC-K1 (phosphodiester compound of vitamin E and vitamin C)	Hirano <i>et al.</i> , 1998
Epigallocatechin-3-gallate (EGCG; green tea polyphenols)	Lin and Lin, 1997; Yang <i>et al.</i> , 1998
Ethylene glycol tetraacetic acid (EGTA)	Janssen and Sen, 1999
Gamma-glutamylcysteine synthetase (gamma-GCS)	Manna <i>et al.</i> , 1999b
Glutathione	Cho <i>et al.</i> , 1998; Schreck <i>et al.</i> , 1992b
L-cysteine	Mihm <i>et al.</i> , 1991
Lacidipine	Cominacini <i>et al.</i> , 1997
Manganese superoxide dismutase (Mn-SOD)	Manna <i>et al.</i> , 1998
Melatonin	Gilad <i>et al.</i> , 1998; Mohan <i>et al.</i> , 1995
N-acetyl-L-cysteine (NAC)	Schreck <i>et al.</i> , 1991
Nordihydroguaiaritic acid (NDGA)	Brennan and O'Neil, 1998; Israël <i>et al.</i> , 1992; Schulze-Osthoff <i>et al.</i> , 1993; Staal <i>et al.</i> , 1993
Orthophenanthroline	Schreck <i>et al.</i> , 1992b
Phenylarsine oxide (PAO, tyrosine phosphatase inhibitor)	Arbault <i>et al.</i> , 1998
Pyrolinedithiocarbamate (PDTC)	Schreck <i>et al.</i> , 1992a
Quercetin	Musonada and Chipman, 1998
Rotenone	Schulze-Osthoff <i>et al.</i> , 1993
S-allyl-cysteine (SAC, garlic compound)	Geng <i>et al.</i> , 1997
Teboxaline (5-(4-chlorophenyl)-N-hydroxy-(4-methoxyphenyl)-N-methyl-1H-pyrazole-3-propanamide)	Kazmi <i>et al.</i> , 1995; Ritchie <i>et al.</i> , 1995
Vitamin C	Staal <i>et al.</i> , 1993
Vitamin E derivatives	Suzuki and Packer, 1993a
α -torphryl succinate	Staal <i>et al.</i> , 1993; Suzuki and Packer, 1993b
α -torphryl acetate	Suzuki and Packer, 1993a
PMC (2,2,5,7,8-pentamethyl-6-hydroxychromane)	Suzuki and Packer, 1993a

Table 2a Proteasome and proteases inhibitors that inhibit Rel/NF- κ B

<i>Molecule</i>	<i>References</i>
<i>Proteasome inhibitors</i>	
Peptide Aldehydes:	Palombella <i>et al.</i> , 1994; Grisham <i>et al.</i> , 1999; Jobin <i>et al.</i> , 1998a
ALLnL (N-acetyl-leuciny-leucynil-norleucynal, MG101)	
LLM (N-acetyl-leuciny-leucynil-methional)	
Z-LLnV (carbobenzoxy-leuciny-leucynil-norvalinal, MG115)	
Z-LLL (carbobenzoxy-leuciny-leucynil-leucynal, MG132)	
Lactacystine, β -lactone	Fenteany <i>et al.</i> , 1998; Grisham <i>et al.</i> , 1999
Boronic acid peptide	Grisham <i>et al.</i> , 1999; Iqbal <i>et al.</i> , 1995
Ubiquitin ligase inhibitors	Yaron <i>et al.</i> , 1997
Cyclosporin A	Frantz <i>et al.</i> , 1994; Kunz <i>et al.</i> , 1994; Marienfield <i>et al.</i> , 1997; McCaffrey <i>et al.</i> , 1994; Meyer <i>et al.</i> , 1997; Wechsler <i>et al.</i> , 1994
FK506 (Tacrolimus)	Okamoto <i>et al.</i> , 1994; Venkataraman <i>et al.</i> , 1995, 1996
Deoxyspergualin	Tepper <i>et al.</i> , 1995
<i>Protease inhibitors</i>	
APNE (N-acetyl-DL-phenylalanine-b-naphthylester)	Higuchi <i>et al.</i> , 1995
BTEE (N-benzoyl L-tyrosine-ethylester)	Rossi <i>et al.</i> , 1998
DCIC (3,4-dichloroisocoumarin)	D'Acquisto <i>et al.</i> , 1998
DFP (diisopropyl fluorophosphate)	
TPCK (N- α -tosyl-L-phenylalanine chloromethyl ketone)	
TLCK (N- α -tosyl-L-lysine chloromethyl ketone)	

Table 2b I κ B ζ phosphorylation and/or degradation inhibitors

<i>Molecule</i>	<i>Inhibit IκBζ's</i>	<i>References</i>
Aspirin, sodium salicylate	Phosphorylation	Frantz and O'Neill, 1995; Kopp and Ghosh, 1994; Yin <i>et al.</i> , 1998
BAY-117821 (E3[(4-methylphenyl)-sulfonyl]-2-propenenitrile)	Phosphorylation	Pierce <i>et al.</i> , 1997
BAY-1170813 (E3[(4-t-butylphenyl)-sulfonyl]-2-propenenitrile)	Phosphorylation	Pierce <i>et al.</i> , 1997
Cycloepoxydon; 1-hydroxy-2-hydroxymethyl-3-pent-1-enylbenzene	Phosphorylation	Gehrt <i>et al.</i> , 1997
Extensively oxidized low density lipoprotein (ox-LDL), 4-Hydroxynonenal (HNE)	Phosphorylation	Brand <i>et al.</i> , 1997; Page <i>et al.</i> , 1999
Ibuprofen	Phosphorylation	Palayoor <i>et al.</i> , 1998
Nitric oxide (NO)	Phosphorylation	Katsuyama <i>et al.</i> , 1998; Matthews <i>et al.</i> , 1996; Spiecker and Liao, 1999
Prostaglandin A1	Phosphorylation	Rossi <i>et al.</i> , 1997
Sanguinarine (pseudochelelythrine, 13-methyl-[1,3]-benzodioxolo- [5,6-c]-1,3-dioxolo-4,5 phenanthridinium)	Phosphorylation	Chaturvedi <i>et al.</i> , 1997
Sulfasalazine	Phosphorylation	Wahl <i>et al.</i> , 1998
Sulindac	Phosphorylation	Yamamoto <i>et al.</i> , 1999
YopJ (encoded by Yersinia pseudotuberculosis)	Phosphorylation	Schesser <i>et al.</i> , 1998
α -melanocyte-stimulating hormone (α -MSH)	Degradation	Manna and Aggarwal, 1998b
β -lapachone	Degradation	Manna <i>et al.</i> , 1999a
Capsaicin (8-methyl-N-vanillyl-6-nonenamide)	Degradation	Singh <i>et al.</i> , 1996b
Core protein of Hepatitis C virus (HCV)	Degradation	Shrivastava <i>et al.</i> , 1998
Diamide (tyrosine phosphatase inhibitor)	Degradation	Toledano and Leonardi, 1991; Singh and Aggarwal, 1995a
Emodin (3-methyl-1,6,8-trihydroxyanthraquinone)	Degradation	Kumar <i>et al.</i> , 1998
Erbstatin (tyrosine kinase inhibitor)	Degradation	Natarajan <i>et al.</i> , 1998
Estrogen (E2)	Degradation	Sun <i>et al.</i> , 1998
Fungal gliotoxin	Degradation	Pahl <i>et al.</i> , 1996
Genistein (tyrosine kinase inhibitor)	Degradation	Natarajan <i>et al.</i> , 1998
IL-13	Degradation	Manna and Aggarwal, 1998a
Leflunomide metabolite (A77 1726)	Degradation	Manna and Aggarwal, 1999
Pervanadate (tyrosine phosphatase inhibitor)	Degradation	Singh and Aggarwal, 1995a; Singh <i>et al.</i> , 1996a
Phenylarsine oxide (PAO, tyrosine phosphatase inhibitor)	Degradation	Mahboubi <i>et al.</i> , 1998; Singh and Aggarwal, 1995a
Resiniferatoxin	Degradation	Singh <i>et al.</i> , 1996b
Sesquiterpene lactones (parthenoide)	Degradation	Hehner <i>et al.</i> , 1998

Lactacystin and its synthetic precursor, β -lactone, represent a second class of inhibitors of the proteasome. These molecules irreversibly block proteasome activity by acylating a threonine residue in the active site of the mammalian proteasome subunit X (Fenteany and Shreiber, 1998; Grisham, *et al.*, 1999). For this reason, lactacystin is considered to be a more

specific inhibitor of the proteasome than the aldehyde peptides.

A third class of proteasome inhibitors is comprised of boronic acid peptides (or dipeptide boronates) named PS-262, PS-402, PS-341 or PS-273, etc. These molecules were originally used as inhibitors of serine proteases, but were also found to act as proteasome

inhibitors and to be more potent than are their aldehyde analogs (Grisham *et al.*, 1999; Iqbal *et al.*, 1995).

An indirect method of blocking proteasome-mediated degradation of I κ B is by inhibiting the ubiquitin ligase that acts on I κ B. For example, Yaron *et al.* (1997) were able to block TNF α -induced degradation of I κ B α by microinjecting phosphopeptides corresponding to the signal-dependent phosphorylation site of I κ B α .

Several serine proteases inhibitors with chymotrypsin-like specificity (DCIC, TPCK, TLCK, BTEE, APNE) are also able to block proteasome function. However, unlike other protease inhibitors, those serine protease inhibitors block I κ B phosphorylation as well as degradation, suggesting that a proteolytic step could (in some cases) be necessary for IKK activation. However, it is important to note that not all serine protease inhibitors are able to inhibit induction of NF- κ B (D'Acquisto *et al.*, 1998; Higuchi *et al.*, 1995; Rossi *et al.*, 1998).

Blockers of I κ B phosphorylation/degradation

In addition to the proteasome inhibitors, several other molecules inhibit NF- κ B by maintaining a high level of I κ B proteins in the cytoplasm and thereby preventing NF- κ B nuclear translocation. Among these molecules, some inhibit I κ B α phosphorylation and its subsequent ubiquitination, while others block I κ B α degradation but their level of action is not known. Those molecules can be grouped into two classes: (1) natural molecules, that is, molecules produced by cells under specific conditions or synthesized by diverse organisms (plants or micro-organisms), and (2) artificial compounds, designed and produced by chemical, pharmacological or molecular biological processes. Most of the molecules mentioned here are cited in Table 2b.

Signaling molecules as blockers of I κ B phosphorylation/degradation

One class of I κ B metabolism blockers includes signaling molecules such as nitric oxide (NO) (Matthews *et al.*, 1996), Extensively Oxidized Low Density Lipoprotein (ox-LDL) (Brand *et al.*, 1997), 4-hydroxynonenal (HNE) (Page *et al.*, 1999), estrogen (E2) (Sun *et al.*, 1998), and prostaglandin A (Rossi *et al.*, 1997). At least one of these inhibitors shows pathway-specific inhibition of NF- κ B; HNE can inhibit LPS-, IL-1- and phorbol ester-induced activation of NF- κ B, but not that induced by TNF α (Page *et al.*, 1999).

Several endogenous intercellular signaling molecules have anti-inflammatory effects that appear to act by blocking Rel/NF- κ B activation. For example, IL-13 can suppress TNF α production by preventing nuclear translocation of the RelA subunit and degradation of I κ B α (Manna and Aggarwal, 1998a). IL-13 also inhibits NF- κ B activation induced by LPS, okadaic acid, hydrogen peroxide, and ceramide (Manna and Aggarwal, 1998a). Alpha-melanocyte-stimulating hormone (α -MSH) can also act as a blocker of NF- κ B activation as induced by a variety of agents (Manna and Aggarwal, 1998b).

Plant anti-inflammatory and anti-tumor molecules

Members of this class of I κ B degradation blockers were generally selected for their anti-inflammatory action or anti-tumor activity, and these are represented by several molecules isolated from plants. For example, this class includes sanguinarine, emodin, and sesquiterpene lactones. Sanguinarine (pseudocheilerythrine, a benzophenanthridine alkaloid) is an anti-tumor agent, which is known to inhibit protein kinase C. However, sanguinarine can also show specific effects on Rel/NF- κ B signaling: that is, it can prevent phosphorylation and degradation of I κ B α in response to TNF α , phorbol ester, IL-1 or okadaic acid stimulation, but not after induction of NF- κ B by ceramide or hydrogen peroxide (Chaturvedi *et al.*, 1997). Similar to sanguinarine, emodin can inhibit a variety of kinases, including protein kinase C, c-Src, p56-lck and HER-2. Moreover, emodin can inhibit I κ B α degradation after stimulation with TNF α (Kumar *et al.*, 1998). Based on its ability to inhibit other kinases, emodin may act directly on the IKK complex to block phosphorylation of I κ B α .

Rel/NF- κ B inhibitors encoded by bacteria or viruses

Several proteins encoded by micro-organisms or viruses have been shown to inhibit NF- κ B, and this can either enhance their replication or contribute to their pathogenicity. The YopJ protein is a Src homology 2 (SH2) domain protein encoded by the enteropathogen *Yersinia pseudotuberculosis*. YopJ inhibits NF- κ B activation by preventing the phosphorylation and degradation of I κ B α . Consequently, eukaryotic cells infected with YopJ-expressing *Yersinia* become impaired in NF- κ B-dependent cytokine expression, which coincides with YopJ-dependent induction of apoptosis (Schesser *et al.*, 1998). Recently, YopJ has been shown to bind directly to IKK β *in vitro* and *in vivo* (Orth *et al.*, 1999).

The core protein of hepatitis C virus (HCV) regulates cellular growth and expression from a number of cellular promoters. Stable cell transfectants expressing the HCV core protein show suppressed TNF α -, okadaic acid-, phorbol ester- and hydrogen peroxide-induced NF- κ B activation through inhibition of I κ B α degradation (Shrivastava *et al.*, 1998). Gliotoxin, a fungal metabolite produced by *Aspergillus fumigatus* is lethal at relatively low concentrations, but it has also been used for its immunosuppressive effects. Gliotoxin blocks activation of NF- κ B DNA, apparently by preventing I κ B α degradation (Pahl *et al.*, 1996).

The African swine fever virus (ASFV) has found a direct way to maintain NF- κ B in the cytoplasm. That is, ASFV encodes an I κ B-like protein that does not have the classic serine residues that direct signal-induced phosphorylation and degradation. Thus, the ASFV-encoded I κ B can interact with p65, inhibit NF- κ B DNA binding, κ B-dependent transcription, and NF- κ B activation by TNF α , IFN- γ and PMA (Powell *et al.*, 1996; Revilla, *et al.*, 1998).

Artificial blockers of I κ B phosphorylation/degradation

Many artificial compounds have been shown to block activation of NF- κ B pathway at the level of I κ B phosphorylation/degradation. Two pharmacologic

small molecules (BAY-117821 and BAY-117083) can inhibit TNF α -induced surface expression of ICAM-1, VCAM-1, and E-selectin (all target genes of NF- κ B). Each can selectively and irreversibly block TNF α -induced phosphorylation of I κ B α (Pierce *et al.*, 1997). However, it is not known what step in NF- κ B activation is blocked by BAY-117821 or BAY-117083.

Cycloepoxydon and 1-hydroxy-2-hydroxymethyl-3-pent-1-enylbenzene are two compounds that inhibit phorbol ester-induced NF- κ B and AP-1-mediated transcription of a reporter gene (Gehrt *et al.*, 1998). Furthermore, in tissue culture cells, cycloepoxydon strongly reduces phorbol ester- and TNF α -induced phosphorylation and degradation of I κ B α (Gehrt *et al.*, 1998).

Genistein and erbstatin are anti-cancer agents, which have been shown to be potent inhibitors of protein tyrosine kinase (PTK) activity. Treatment of human myeloid U937 cells, T or B cells with either inhibitor completely suppresses TNF α -induced NF- κ B activation by blocking I κ B α degradation and the consequent translocation of the p50 subunit without affecting p50 or c-Rel (Natarajan *et al.*, 1998). In addition, NF- κ B activation after infection with Newcastle Disease Virus can be blocked by genistein (Umansky *et al.*, 1996). However, genistein and erbstatin do not block NF- κ B induction in all cells. For example, in MCA-101 fibrosarcoma cells and in human alveolar epithelial A439 cells, genistein and erbstatin failed to block activation of NF- κ B (Bergmann *et al.*, 1998; Mahboubi *et al.*, 1998).

Down-regulation of NF- κ B nuclear functions: nuclear translocation, DNA binding and transcriptional activation

Direct inhibition of NF- κ B-specific transactivation could involve blocking either nuclear translocation, DNA binding, or transactivation by NF- κ B dimers. In many studies described below, these last steps of NF- κ B activation have been used as indicators of NF- κ B inhibition (either as reduced binding in an EMSA or reduced κ B site reporter gene activity), but that does not mean that an earlier step is not the direct target of the inhibitor. For example, the inhibition process could also occur by maintaining a pool of I κ B proteins in the cytoplasm. As in the previous section, molecules cited below and in Table 3 belong to two major groups, depending on whether they are natural or artificial.

Up-regulation of I κ B

A few molecules have been found to inhibit NF- κ B by up-regulating I κ B expression. One such molecule is the β -amyloid peptide, which is deposited in the neuritic plaques that are a characteristic feature of Alzheimer's disease (AD). β -amyloid peptide appears to show a cell type-specific effect on NF- κ B, acting as an inhibitor in some cells and an activator in others. The constitutive NF- κ B activity present in fetal rat cortical neurons decreases in a concentration- and time-dependent fashion following exposure to β -amyloid, without any corresponding decrease in RelA mRNA or protein (Bales *et al.*, 1998). However, both I κ B α mRNA and

protein are increased in these cells following treatment with β -amyloid, and this increase is likely to be responsible for the decrease in activated NF- κ B. This hypothesis is supported by the finding that pretreatment of cortical cultures with an antisense oligonucleotide to I κ B α mRNA is neuroprotective towards β -amyloid toxicity. In contrast to cortical neurons, exposure of rat primary astroglial cultures to β -amyloid peptide results in a concentration- and time-dependent activation of NF- κ B with the subsequent increased transcription of the NF- κ B target genes encoding IL-1 β and IL-6. These data suggest that β -amyloid-induced neurotoxicity as well as astrocyte activation may be mediated by Rel/NF- κ B proteins, and thus alterations in NF- κ B-directed gene expression may contribute to both the neurodegeneration and inflammatory response which occur in AD (Bales *et al.*, 1998).

Among cytokines, IL-10 and IL-13 (which have powerful anti-inflammatory activities *in vitro* and *in vivo*) both suppress nuclear localization of NF- κ B and augment I κ B α mRNA expression (Ehrlich *et al.*, 1998; Lentsch *et al.*, 1997). IL-11 is also able to block NF- κ B activation by increasing mRNA and protein levels of I κ B α and I κ B β following LPS treatment of macrophages (Trepicchio and Dorner, 1998).

Inhibitors of Rel/NF- κ B nuclear transport

Certain Rel/NF- κ B inhibitors block nuclear transport of the transcription factor. One such approach has used cell-permeable peptides that contain the nuclear localizing sequence of p50. These peptides are thought to inhibit nuclear translocation of p50-containing dimers by saturating the nuclear import machinery responsible for their uptake (Lin *et al.*, 1995). An allosteric drug, o,o'-bismyristoyl thiamine disulfide (BMT), suppresses HIV-1 replication through prevention of nuclear translocation of both HIV-1 Tat and NF- κ B (Shoji *et al.*, 1998).

Inhibitors of Rel/NF- κ B DNA binding and transactivation

The largest class of non-specified NF- κ B inhibitors affect DNA binding and κ B site-dependent gene expression. However, for several of the molecules cited below and in Table 3, inhibition of DNA binding is not necessarily meant to indicate that DNA binding *per se* is impaired, only that the effect of the inhibitor on NF- κ B has been measured by assaying the amount of protein able to bind a κ B site probe in an EMSA, as compared to control cells. Some of the inhibitors included in this section are natural products like Vascular Endothelial Growth Factor (VEGF) which is produced by almost all tumors and affects the ability of hemopoietic progenitor cells to differentiate into dendritic cells (Gabrilovich *et al.*, 1998; Oyama *et al.*, 1998).

Some of these molecules show stimulus-dependent inhibition of NF- κ B activation. For example, the bioflavonoid silymarin potently suppresses NF- κ B DNA-binding activity induced by okadaic acid or by LPS in the hepatoma cell line HepG2, but TNF α -induced NF- κ B activation is not affected by silymarin (Saliou *et al.*, 1998).

Some of these molecules have therapeutic potential. For example, the atrial natriuretic peptide (ANP) can prevent the marked augmentation of NF- κ B DNA-binding activity which is associated with rat liver damage during reperfusion (Gerbes *et al.*, 1998). In addition, KT-90 (a synthesized morphine derivative) is an analgesic, which is five times more potent than morphine. KT-90 has also been found to inhibit the growth of certain human cancer cell lines up to 80 times more potently than morphine. KT-90 inhibits TNF α gene expression induced by okadaic acid and phorbol esters, and is associated with a reduction in NF- κ B DNA-binding activity (Sueoka *et al.*, 1998). Ribavirin and pentoxifylline, inhibitors of viral production, have been reported to block NF- κ B

DNA binding (Biswas *et al.*, 1993; Fiedler *et al.*, 1996). Pentoxifylline is particularly interesting in that it appears to have specific effects on c-Rel (among Rel/NF- κ B family members) and to block c-Rel in a cell type-specific fashion, namely, in T cells but not in B cells (Wang *et al.*, 1997). Lastly, this group includes two anti-inflammatory neuropeptides, vasoactive intestinal peptide (VIP) and pituitary adenylate cyclase-activating polypeptide (PACAP), which can inhibit TNF α production (by blocking induction of NF- κ B) in LPS-stimulated macrophages (Delgado *et al.*, 1998).

Several small molecules, with quite defined activities, have been shown to block NF- κ B activity. D609, RO31-8220 and SB203580 are compounds that selectively inhibit phosphatidylcholine-phospholipase C inhibitor,

Table 3 Miscellaneous inhibitors of NF- κ B

<i>Inhibitor molecule</i>	<i>Detected effect</i>	<i>References</i>
β -amyloid protein	I κ B α upregulation	Bales <i>et al.</i> , 1998
Glucocorticoids (dexamethasone, prednisone, methylprednisolone)	I κ B α upregulation	Auphan <i>et al.</i> , 1995; Brostjan <i>et al.</i> , 1996; Ray and Prefontaine, 1994; Scheinman <i>et al.</i> , 1995
IL-10	I κ B α upregulation	Ehrlich <i>et al.</i> , 1998; Lentsch <i>et al.</i> , 1997
IL-13	I κ B α upregulation	Ehrlich <i>et al.</i> , 1998; Lentsch <i>et al.</i> , 1997; Manna and Aggarwal, 1998a
IL-11	I κ B α , I κ B β upregulation	Trepicchio and Dorner, 1998
Leptomycin B (LMB)	nuclear transport	Rodriguez <i>et al.</i> , 1999
NLS cells permeable peptides	nuclear transport	Lin <i>et al.</i> , 1995
o,o'-bismyristoyl thiamine disulfide (BMT)	nuclear transport	Shoji <i>et al.</i> , 1998
ADP ribosylation inhibitors (nicotinamide, 3-aminobenzamide)	DNA binding	Le Page <i>et al.</i> , 1998
Atrial natriuretic peptide (ANP)	DNA binding	Gerbes <i>et al.</i> , 1998
Atrovastatin (HMG-CoA reductase inhibitor)	DNA binding	Bustos <i>et al.</i> , 1998; Hernandez-Presa <i>et al.</i> , 1998
Calcitriol (1 α ,25-dihydroxyvitamine D3)	DNA binding	Harant <i>et al.</i> , 1998
Diamide	DNA binding	Toledano and Leonard, 1991
E3330 (quinone derivative)	DNA binding	Hiramoto <i>et al.</i> , 1998; Kimura <i>et al.</i> , 1997
Glycyrrhizin	DNA binding	Wang <i>et al.</i> , 1998
Herbimycin A	DNA binding	Iwasaki <i>et al.</i> , 1992; Mahon and O'Neill, 1995
Hypericin	DNA binding	Bork <i>et al.</i> , 1999
Hydroquinone (HQ)	DNA binding	Pyatt <i>et al.</i> , 1998b
IL-4	DNA binding	Manna and Aggarwal, 1998c
I κ B-like proteins (encoded by ASFV)	DNA binding	Powell <i>et al.</i> , 1996; Revilla <i>et al.</i> , 1998
KT-90 (morphine synthetic derivatives)	DNA binding	Sueoka <i>et al.</i> , 1998
Metals (chromium, cadmium, gold, mercury zinc, arsenic)	DNA binding	Shumilla <i>et al.</i> , 1998; Yang <i>et al.</i> , 1995
Mevinolin, 5'-methylthioadenosine (MTA)	DNA binding	Law <i>et al.</i> , 1992
N-ethyl-maleimide (NEM)	DNA binding	Toledano and Leonard, 1991
Nicotinic	DNA binding	Sugano <i>et al.</i> , 1998
Pentoxifylline (1-(5'-oxohexyl) 3,7-dimethylxanthine, PTX)	DNA binding	Biswas <i>et al.</i> , 1993; Wang <i>et al.</i> , 1997
Phenyl-N-tert-butylnitron (PBN)	DNA binding	Kotake <i>et al.</i> , 1998
Pituitary adenylate cyclase-activating polypeptide (PACAP)	DNA binding	Delgado <i>et al.</i> , 1998
Pyrithione	DNA binding	Kim <i>et al.</i> , 1999
Quinadril (ACE inhibitor)	DNA binding	Bustos <i>et al.</i> , 1998; Hernandez-Presa <i>et al.</i> , 1998
Ribavirin	DNA binding	Fiedler <i>et al.</i> , 1996
Secretory leukocyte protease inhibitor (SLPI)	DNA binding	Jin <i>et al.</i> , 1997
Serotonin derivative (N-(p-coumaroyl) serotonin, SC)	DNA binding	Kawashima <i>et al.</i> , 1998
Silymarin	DNA binding	Saliou <i>et al.</i> , 1998
Sulfasalazine	DNA binding	Egan and Sandborn, 1998
Vascular endothelial growth factor (VEGF)	DNA binding	Oyama <i>et al.</i> , 1998; Gabrilovich <i>et al.</i> , 1998
Vasoactive intestinal peptide (VIP)	DNA binding	Delgado <i>et al.</i> , 1998
D609 (phosphatidylcholine-phospholipase C inhibitor)	Transactivation	Bergmann <i>et al.</i> , 1998
Glucocorticoids (dexametasone, prednisone, methylprednisolone)	Transactivation	Auphan <i>et al.</i> , 1995; Brostjan <i>et al.</i> , 1996; Ray and LevPrefontaine, 1994; Scheinman <i>et al.</i> , 1995
RO31-8220 (PKC inhibitor)	Transactivation	Bergmann <i>et al.</i> , 1998
SB203580 (p38 MAPK inhibitor)	Transactivation	Bergmann <i>et al.</i> , 1998
Triptolide (PG490, extract of Chinese herb)	Transactivation	Qiu <i>et al.</i> , 1999
LY294,002	RelA phosphorylation and transactivation	Sizemore <i>et al.</i> , 1999
Mesalamine	transactivation	Egan <i>et al.</i> , 1999
Wortmannin (fungal metabolite)	Transactivation	Reddy <i>et al.</i> , 1997

protein kinase C and p38 MAPK, respectively. In the human A549 cell line, they are also able to block NF- κ B-dependent transcription after stimulation by IL-1 and TNF α . However, none of these molecules inhibits I κ B α degradation, NF- κ B nuclear translocation or DNA binding (Bergmann *et al.*, 1998). In another study, LY294,002 and wortmannin (both PI-3 kinase inhibitors) have been used to block IL-1-induced NF- κ B activation of a reporter gene (Reddy *et al.*, 1997; Sizemore *et al.*, 1999). LY294,002 could not inhibit I κ B α degradation or NF- κ B DNA binding, but it could block IL-1-stimulated phosphorylation of NF- κ B, especially the p65/RelA subunit. Furthermore, overexpression of the p110 catalytic subunit of PI-3 kinase induces RelA-mediated transactivation and LY294,002 represses this process (Sizemore *et al.*, 1999).

Lastly, there have been two reports suggesting that Bcl-2, an inhibitor of apoptosis, can also function as an NF- κ B inhibitor. In one study (Grimm *et al.*, 1996), Bcl-2 suppressed transactivation by RelA without affecting the nuclear transport or DNA binding of NF- κ B; whereas in a second report (Badrichani *et al.*, 1999), Bcl-2 blocked degradation of I κ B α .

Commonly used drugs that act, at least in part, by inhibiting NF- κ B activity

Because many of NF- κ B target genes are involved in the acute phase or inflammatory response, inhibition of NF- κ B has been used to minimize the pathologic effects induced by the immune system misregulation. As such, several of the most commonly used drugs can inhibit NF- κ B activity.

Anti-inflammatory drugs

One example of an anti-inflammatory agent that can inhibit NF- κ B and whose molecular target appears to be known is aspirin or sodium salicylate (Grilli *et al.*, 1997). Aspirin is one of a class of nonsteroidal anti-inflammatory drugs (NSADs) that have commonly been thought to act via inhibition of prostaglandin synthesis (Weissmann, 1991). However, aspirin has also been shown to block NF- κ B activity by preventing I κ B α degradation after stimulation by phorbol ester, TNF α or LPS (Kopp and Ghosh, 1994). Even if the specificity of aspirin for inhibition of NF- κ B has been

Table 4 Inhibition of specific NF- κ B activating stimuli

<i>TNFα</i>	<i>IL-1</i>	<i>Phorbol esters</i>	<i>Lipopolysaccharide</i>
Anti-oxidants	Anti-oxidants	Anti-oxidants	Anti-oxidants
Moast proteasome inhibitors	Most proteasome inhibitors	Most proteasone inhibitors	Most proteasome inhibitors
Aspirin, Sodium salicylate	Calcitrol	Capsaicin	ADP ribosylation inhibitors
α -melanocyte-stimulating hormone	D609	Core protein of hepatitis C virus	Aspirin, Sodium salicylate
BAY-117821, BAY-117083	Diamide	Cyclosporin A	α -melanocyte-stimulating hormone
β -lapachone	Fungal gliotoxin	Cycloepoxydon; 1-hydroxy-2-hydroxymethyl-3-Pent-1-enylbenzene	β -lapachone
Calcitrol	Herbimycin A	Diamide	Cyclosporin A
Capsaicin	Leptomycin B	E3330	Deoxyspergualin
Core protein of hepatitis C virus	LY294,002	Estrogen	FK506
Cycloepoxydon; 1-hydroxy-2-hydroxymethyl-3-pent-1-enylbenzene	Mesalamine	FK506	Fungal gliotoxin
D609	Pervanadate	Fungal gliotoxin	Glucocorticoids
Diamide	Phenylarsine oxide	Glucocorticoids	IL-10, IL-11, IL-13
E3330	RO31-8220	Herbimycin A	Mevinolin, 5'-methylthioadenosine
Emodin	Sanguinarine	KT-90	Leflunomide
Erbstatin, Genistein	Wortmannin	Leflunomide	Nicotine
Fungal gliotoxin	4-Hydroxynonenal	o,o'-bismyristoyl thiamine disulfide	NLS cell permeable peptides
Glucocorticoids		Pentoxifylline	N-substituted benzamide
Hydroquinone		Pervanadate	Phenyl-N-tert-butylnitron
Hypericin		Phenylarsine oxide	Pituitary adenylate cyclase-activating polypeptide
I κ B-like proteins		Pituitary adenylate cyclase-activating polypeptide	Silymarin
Ibuprofen		Prostaglandine A1	Secretory leukocyte protease inhibitor
IL-4, IL-10, IL-13		Sanguinarine	Sulfasalazine
KT-90		Sesquiterpene lactones	Vasoactive intestinal peptide
Leflunomide		Sulfasalazine	4-Hydroxynonenal
Leptomycin B		TLCK, TPCK, DCIC	
NLS cell permeable peptides		4-Hydroxynonenal	
Pervanadate			
Phenylarsine oxide			
Pituitary adenylate cyclase-activating polypeptide			
Pentoxifylline			
Prostaglandin A1			
Resiniferatoxin			
Rotenone			
RO31-8220			
SB203580			
Sanguinarine			
Sesquiterpene lactones			
Sulfasalazine			
Sulindac			
TLCK, TPCK, DCIC			
Triptolide			
Vascular endothelial growth factor			

disputed (Frantz and O'Neill, 1995), it is a quite effective blocker of NF- κ B induction. Aspirin and sodium salicylate specifically inhibit NF- κ B nuclear translocation by preventing I κ B α phosphorylation and degradation (Pierce *et al.*, 1996). Recently, Yin *et al.* (1998) have shown that aspirin can directly bind to and inhibit the kinase activity of IKK β by reducing its ability to bind ATP. Other NSAIDs, such as tepoxaline, deferoxamine, and ibuprofen, are also able to inhibit NF- κ B (Kazmi *et al.*, 1995; Palayoor *et al.*, 1998; Ritchie *et al.*, 1995). Of particular interest, ibuprofen has been used as a growth suppressor for human colon cancer and to sensitize prostate cancer cells to ionizing therapy (Palayoor *et al.*, 1998).

Glucocorticoids, such as dexametasone, prednisone and methylprednisolone, are used for their anti-inflammatory properties and to prevent allograft rejection. Their physiological effects are, at least partially, mediated through inhibition of NF- κ B. Two models have been proposed for inhibition of NF- κ B by glucocorticoids. On the one hand, glucocorticoids have been shown to inhibit κ B-dependent transcription by directly interacting with the NF- κ B RelA subunit and affecting its transactivation potential (Brostjan *et al.*, 1996; De Bosscher *et al.*, 1997; Ray and Prefontaine, 1994; Simpson and Morris 1999). On the other hand, glucocorticoids have also been shown to enhance I κ B α production, leading to sustained inhibition of NF- κ B (Auphan *et al.*, 1995; Scheinman *et al.*, 1995).

Immunosuppressive agents

Several well-known immunosuppressants target NF- κ B. Cyclosporin A (CsA), inhibits B- and T-cell proliferation by blocking the activity of calcineurin, a calcium and calmodulin-dependent serine/threonine phosphatase (Frantz *et al.*, 1994). Several reports have shown that CsA can also inhibit NF- κ B induction, although the mechanism by which CsA exerts this inhibitory effect may differ in different situations. *In vitro*, CsA has been reported to act as a non-competitive inhibitor of the chymotrypsin-like activity of the proteasome, enabling it to block LPS-induced I κ B degradation and p105 processing *in vivo* (Meyer *et al.*, 1997). Similarly, CsA prevents NF- κ B nuclear translocation in stimulated T cells (McCaffrey *et al.*, 1994) by preventing the inducible degradation of I κ B α and I κ B β ; however, in these cells, CsA does not inhibit the processing of p105 to p50 (Marienfeld *et al.*, 1997). CsA is able to block the activation of IL-8 gene expression by NF- κ B in T cells (Wechsler *et al.*, 1994) and it reduces NF- κ B DNA binding in murine mesangial cells (Kunz *et al.*, 1995). Interestingly, like aspirin, CsA has also been shown to be neuroprotective (Meyer *et al.*, 1997).

FK506 is an immunosuppressant that acts as a potent blocker of B- and T-cell proliferation. At least in part, FK506, like CsA, acts by blocking the activity of calcineurin. However, unlike CsA, the inhibitory effect of FK506 on NF- κ B appears to be specific for c-Rel, among the Rel/NF- κ B family members. That is, FK506 can specifically block c-Rel nuclear translocation (but not p50/RelA) after treatment of cells with phorbol esters and ionomycin (Venkataraman *et al.*, 1995, 1996; Sen *et al.*, 1995). FK506 also has been reported to block NF- κ B DNA

binding in Jurkat T cells stimulated with phorbol ester (Okamoto *et al.*, 1994). Therefore, the anti-proliferative effects of FK506 in T cells results from inhibition of Rel/NF- κ B, which consequently blocks transcription of the IL-2 and IL-2 receptor genes by interfering with the induction of κ B site-dependent transcription of their promoters (Serfling *et al.*, 1995; Venkataraman *et al.*, 1995).

Certain other immunosuppressants act at different levels than CsA and FK506 to block immune cell proliferation. For example, PG490 (pure triptolide, a diterpene triepoxide) is an immunosuppressant molecule that can synergize with CsA to inhibit transcriptional activation by NF- κ B. However, PG490 does not appear to interfere with the induction of NF- κ B DNA binding, suggesting that it acts to inhibit transcriptional activation by NF- κ B (Qiu *et al.*, 1999). Moreover, the immunosuppressant deoxyspergualin inhibits NF- κ B nuclear translocation by a mechanism involving the heat shock protein hsp70 (Tepper *et al.*, 1995).

Thus, it appears that different immunosuppressants can inhibit NF- κ B by distinct mechanisms. They can inhibit NF- κ B nuclear translocation by stabilizing I κ B α (Meyer *et al.*, 1997), by inhibiting calcineurin (Frantz *et al.*, 1994), by binding heat shock proteins (Tepper *et al.*, 1995) or by modulating the DNA binding or transactivation potential of NF- κ B (Kunz *et al.*, 1995; McCaffrey *et al.*, 1994; Qiu *et al.*, 1999; Wechsler *et al.*, 1994).

Molecular blockers of the NF- κ B signaling pathway

Another general strategy for inhibiting NF- κ B activity is to develop genetically-engineered proteins that block specific steps in the activation process. Because many of the molecular details of the NF- κ B signaling pathway are now known, it has been relatively straight-forward to design constitutive inhibitors of molecules in this pathway. The effects of these polypeptide inhibitors are then determined in tissue culture cells or occasionally in transgenic mice. At this time, these types of inhibitors have not yet been used in clinical studies. In part, this is because these experimental approaches have sometimes yielded different results depending on the experimental approach used (transient transfection vs stable cell lines, overexpression vs knockout, etc). Nevertheless, the characterization of these types of 'molecular' inhibitors may lead to development of very specific anti-NF- κ B drugs. Below are some examples of inhibitors of NF- κ B activity that have been developed based on the structure and function of members of the signaling cascade.

Tumor necrosis factor receptor-associated factors (TRAFs)

Many cytokines signal through distinct cell-surface receptors to activate transcription factor NF- κ B. Members of the tumor necrosis factor receptor-associated factors (TRAF) protein family act as adaptor molecules for the activation of NF- κ B by the TNF receptor superfamily (including TNFR1, TNFR2 and CD40). TRAF2 is recruited to the TNFR1 and TNFR2 receptors following TNF stimulation, and it is required for CD40- and TNF α -mediated activation of NF- κ B. A TRAF2 mutant lacking its N-terminal RING finger

domain is a dominant-negative inhibitor of TNF α -(but not IL-1) induced NF- κ B activation (Hsu *et al.*, 1996). However, cells derived from TRAF2- deficient mice have only a small reduction in NF- κ B activation after TNF α stimulation, suggesting that TRAF2-independent pathways of NF- κ B activation exist (Yeh *et al.*, 1997). In contrast to TRAF2, a dominant-negative mutant of TRAF6 inhibits NF- κ B activation signaled by IL-1 but not by TNF α (Cao *et al.*, 1996), and TRAF6 appears to participate exclusively in the IL-1 signal transduction pathway by interacting with the IL-1 receptor and NIK.

I-TRAF is a TRAF-interacting protein that binds to the conserved TRAF-C domain of the TRAF1, TRAF2, and TRAF3. Overexpression of I-TRAF inhibits TRAF2-mediated NF- κ B activation as signaled by CD40 and both TNF receptors. Thus, I-TRAF appears to act as a natural inhibitor of TRAF function (Rothe *et al.*, 1996).

NIK and MEKK1

Two kinases that can lead to activation of the IKK complex are NIK and MEKK1. Overexpression of NIK potently induces NF- κ B (Malinin *et al.*, 1997), and MEKK1 is activated by TNF α and IL-1 and can potentiate the stimulatory effect of TNF α on IKK and NF- κ B activation (Hirano *et al.*, 1996; Lee *et al.*, 1998).

NIK has also been reported to interact with several members of the NF- κ B signaling pathway, including TRAF2, TRAF6, and IKK. In addition, NIK may directly phosphorylate members of the IKK complex. Overexpression in cells of kinase-deficient NIK mutants not only fails to activate NF- κ B, but blocks the ability of IL-1 and TNF α to induce NF- κ B (Malinin *et al.*, 1997; Song *et al.*, 1997). However, others have questioned the involvement of NIK in activation of NF- κ B by IL-1 and TNF α (Baud *et al.*, 1999), and NIK-deficient mice, despite dying at an early embryonic stage, still show activation of NF- κ B after IL-1 and TNF α stimulation (D Goeddel, personal communication).

When overexpressed, MEKK1 stimulates the activities of both IKK α and IKK β in transfected HeLa cells and directly phosphorylates the IKKs *in vitro* (Lee *et al.*, 1998). Furthermore, MEKK1 may act in parallel to NIK, leading to synergistic activation of the IKK complex (Nemoto *et al.*, 1998), and a dominant-negative mutant of MEKK1 partially blocks activation of the IKK complex by TNF α (Hirano *et al.*, 1996; Lee *et al.*, 1997). However, the physiological relevance of activation of IKK and NF- κ B by overexpressed MEKK1 has been questioned (Karin and Delhase, 1998).

IKK complex mutants

A high molecular weight complex containing IKK α and IKK β is directly responsible for the induced phosphorylation of I κ B α , and overexpression of dominant-negative mutants of IKK can block activation of NF- κ B (Karin, 1999, this issue). However, the two IKKs seem to have different functions in the cell and their dominant-negative mutants do not always behave identically. Dominant-negative mutations in the kinase domain of either IKK suppress IL-1 and TNF α induction of NF- κ B, but IKK β mutants always have

more dramatic effects, and other results suggest that TNF α and IL-1 primarily activate IKK through IKK β (Karin, 1999, this issue). Dominant-negative forms of the IKKs, which are capable of blocking activation of NF- κ B, can be created either by mutations in the ATP-binding site or by mutations in the kinase activation loop (DiDonato *et al.*, 1997; Ling *et al.*, 1998; Mercurio *et al.*, 1997; Regnier *et al.*, 1997; Woronicz *et al.*, 1997; Zandi *et al.*, 1997).

Dominant-negative IKK mutants can show stimulus-dependent inhibition. For example, overexpression of wild-type IKK α , wild-type IKK β or a dominant-negative mutant IKK α does not affect LPS-induced κ B-dependent transcription in THP-1 monocytic cells, whereas a dominant-negative mutant of IKK β does inhibit LPS induction of κ B site-dependent transcription (O'Connell *et al.*, 1998).

The analysis of IKK knockout mice again points out the need for caution in interpreting tissue culture experiments wherein proteins are grossly overexpressed. First, mice with knockouts of IKK α versus IKK β have quite different phenotypes: when the gene encoding IKK α is disrupted, mice die shortly after birth with blocked limb emergence due to abnormal keratinocyte function, whereas IKK β -deficient mice die between embryonic day 12.5 and day 14 with extensive liver damage due to apoptosis (similar to the phenotype observed in mice lacking RelA) (Karin, 1999, this issue). Furthermore, I κ B α degradation and NF- κ B induction are not impaired after induction by TNF α or IL-1 in cells from IKK α -deficient mice, however, TNF α - and IL-1-induced degradation of I κ B α and NF- κ B activation are abrogated in cells from IKK β -deficient mice. Thus, most tissue culture experiments that have looked at IKK activation have focused on signaling through IKK β .

IKK γ is a member of the IKK complex and is required for kinase activation. IKK γ plays a crucial role in NF- κ B activation by IKK since cells that do not express IKK γ do not show induced NF- κ B activity in response to various stimuli (Yamaoka *et al.*, 1998). Moreover, cells lacking IKK γ cannot be transformed by the Tax oncoprotein of HTLV-1, suggesting that IKK γ could be a target for anti-HTLV-1 therapies.

I κ B α super-repressor

Blocking nuclear transport of NF- κ B can also be accomplished by imprisoning it in the cytoplasm in a manner such that it cannot respond to an activation signal. This has been achieved by using mutant forms of I κ B α , called super-repressors, which cannot be phosphorylated or degraded. These super-repressor I κ B α 's carry mutations of the signal-induced phosphorylation sites Ser 32 and 36 (wherein alanine residues replace the 2 serines), of the lysine ubiquitination sites (Lys->Arg mutations) or are deleted for their first 40 amino acids and thus, can be neither phosphorylated nor ubiquitinated. In addition, specific C-terminal Ser to Ala mutations are sometimes included to reduce the constitutive turnover of I κ B α (van Antwerp *et al.*, 1996). Because these super-repressor forms of I κ B α can still interact with NF- κ B dimers, they are very potent inhibitors of its activity by keeping it permanently in the cytoplasm (Bentires-Alj *et al.*,

1999; Jobin *et al.*, 1998b; van Antwerp *et al.*, 1996; Wang *et al.*, 1996). Such molecules have been used successfully to inhibit NF- κ B activity and to study its role in development (Bushdid *et al.*, 1998; Kanegae *et al.*, 1998) or to sensitize cells to apoptosis-inducing agents (van Antwerp *et al.*, 1996; Wang *et al.*, 1996).

Recently, inhibition of NF- κ B through expression of an I κ B α super-repressor has been used to sensitize chemoresistant tumors to apoptosis induced by TNF α and the chemotherapeutic compound CPT-11, resulting in tumor regression (Wang *et al.*, 1999), and also to inhibit the growth of human head and neck carcinoma cells *in vitro* and *in vivo* (Duffey *et al.*, 1999). But again, such results must be taken with caution, in that overexpression of the I κ B α super-repressor has also been associated with the spontaneous development of squamous cell carcinoma in one murine model system (van Hogerlinden *et al.*, 1999).

Conclusions

From the commonly-used aspirin to the I κ B α super-repressor, numerous inhibitors of the NF- κ B activity have been described. Their mode of action is sometimes known, other times it has simply been noticed that such molecules inhibit NF- κ B-induced DNA binding. Some Rel/NF- κ B inhibitors are 'broad-range' inhibitors, such as the many anti-oxidants and proteasome inhibitors, which can block most NF- κ B activating signals. However, the broad-range inhibitors may affect a variety of pathways; for example, AP-1 is also induced by oxidative stress and TNF α . Other inhibitors of NF- κ B block activation when induced by only certain stimuli, for example by acting on a particular protein in the signaling cascade or by blocking specific Rel/NF- κ B complexes. Nevertheless,

even these specific inhibitors may block other pathways, either because their target participates in several intracellular pathways or because they block a certain class of protein. Indeed, in most of the studies described in this review, neither the molecular target in the NF- κ B signaling pathway nor cell-type specificity of the inhibitors are known. In addition, it is often not clear whether the concentrations of inhibitors used in tissue culture experiments can be applied to *in vivo* situations. Lastly, some inhibitors of NF- κ B may have effects on other signaling pathways or cellular functions. Therefore, such inhibitors can perhaps also be used to identify new pathways used by members of the Rel/NF- κ B cascade. The use of overlapping signaling pathways makes it a challenge to find molecules that block specific pathways leading to NF- κ B activation without interfering with other signaling cascades. Recent descriptions of the crystallographic structure of NF- κ B dimers or NF- κ B/I κ B complexes will no doubt help in the design of molecules that are quite specific for inhibiting only Rel/NF- κ B proteins. A future goal will be to discover molecules that can inhibit distinct Rel/NF- κ B complexes induced by select stimuli in specific cell types.

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References

- Arbault S, Edeas M, Legrand-Poels S, Sojic N, Amatore C, Piette J, Best-Belpomme M, Lindenbaum A and Vuillaume M. (1998). *Biomed. Pharmacother.*, **51**, 430–438.
- Auphan N, DiDonato JA, Rosette C, Helmberg A and Karin M. (1995). *Science*, **270**, 286–290.
- Badrichani AZ, Stroka DM, Bilbao G, Curiel DT, Bach FH and Ferran. (1999). *J. Clin. Invest.*, **103**, 543–553.
- Bales KR, Du Y, Dodel RC, Yan GM, Hamilton-Byrd E and Paul SM. (1998). *Brain Res. Mol. Brain Res.*, **57**, 63–72.
- Barkett M and Gilmore TD. (1999). *Oncogene*, **18**, 6910–6924.
- Baud V, Lin Z-G, Bennett B, Suzuki N, Xia Y and Karin M. (1999). *Genes Dev.*, **13**, 1297–1308.
- Bentires-Alj M, Hellin AC, Ameyar M, Chouaib S, Merville MP and Bours V. (1999). *Cancer Res.*, **59**, 811–815.
- Bergmann M, Hart L, Lindsay M, Barnes PJ and Newton R. (1998). *J. Biol. Chem.*, **273**, 6607–6610.
- Biswas DK, Dezube BJ, Ahlers CM and Pardee AB. (1993). *J. AIDS*, **6**, 778–786.
- Bork PM, Bacher S, Schmitz ML, Kaspers U and Heinrich M. (1999). *Planta Med.*, **65**, 297–300.
- Brand K, Eisele T, Kreuzel U, Page M, Page S, Haas M, Gerling A, Kaltschmidt C, Neumann FJ, Mackman N, Baeurele PA, Walli AK and Neumeier D. (1997). *Arterioscler. Thromb. Vascul. Biol.*, **17**, 1901–1909.
- Brennan P and O'Neill LA. (1998). *Biochem. Pharm.*, **55**, 965–973.
- Brostjan C, Anrather J, Csizmadia V, Stroka D, Soares M, Bach FH and Winkler H. (1996). *J. Biol. Chem.*, **271**, 19612–19616.
- Bushdid PB, Brantley DM, Yull FE, Blaeuer GL, Hoffman LH, Niswander L and Kerr LD. (1998). *Nature*, **392**, 615–618.
- Bustos C, Hernandez-Presa MA, Ortego M, Tunon J, Ortega L, Perez F, Diaz C, Hernandez G and Egido J. (1998). *J. Am. Coll. Cardiol.*, **32**, 2057–2064.
- Cao Z, Xiong J, Takeuchi M, Kurama T and Goeddel DV. (1996). *Nature*, **383**, 443–446.
- Chaturvedi MM, Kumar A, Darnay BG, Chainy GBN, Aggarwal S and Aggarwal BB. (1997). *J. Biol. Chem.*, **272**, 30129–30134.
- Cho S, Urata Y, Iida T, Goto S, Yamaguchi M, Sumikawa K and Kondo T. (1998). *Biochem. Biophys. Res. Comm.*, **253**, 104–108.
- Cominacini L, Garbin U, Fratta Pasini A, Paulon T, Davoli A, Campagnola M, Marchi E, Pastorino AM, Gaviraghi G and Lo Cascio V. (1997). *J. Hypertens.*, **5**, 1633–1640.
- D'Acquisto F, Sautebin L, Iuvone T, Di Rosa M and Carnuccio R. (1998). *FEBS Lett.*, **440**, 76–80.

- De Bosscher K, Lienhard Schmitz M, Vanden Berghe W, Plaisance S, Fiers W and Haegeman G. (1997). *Proc. Natl. Acad. Sci. USA*, **94**, 13504–13509.
- Delgado M, Munoz-Elias EJ, Kan Y, Gozes I, Fridkin M, Brenneman DE, Gomariz RP and Ganea D. (1998). *J. Biol. Chem.*, **273**, 31427–31436.
- DiDonato JA, Hayakawa M, Rothwarf DM, Zandi E and Karin M. (1997). *Nature*, **388**, 548–554.
- Duffey DC, Chen Z, Dong G, Ondrey FG, Wolf JS, Brown K, Siebenlist U and Van Waes C. (1999). *Cancer Res.*, **59**, 3468–3474.
- Egan LJ and Sandborn WJ. (1998). *Gastroenterology*, **115**, 1295–1296.
- Egan LJ, Mays DC, Huntoon CJ, Bell MP, Pike MG, Sandborn WJ, Lipsky JJ and McKean DJ. (1999). *J. Biol. Chem.*, **274**, 26448–26453.
- Ehrlich LC, Hu S, Peterson PK and Chao CC. (1998). *Neuroreport*, **9**, 1723–1726.
- Fenteany G and Schreiber SL. (1998). *J. Biol. Chem.*, **273**, 8545–8548.
- Fiedler MA, Wernke-Dollries K and Stark JM. (1996). *J. Virol.*, **70**, 9079–9082.
- Frantz B, Nordby EC, Bren G, Steffan N, Paya CV, Kincaid RL, Tocci MJ, O'Keefe SJ and O'Neill EA. (1994). *EMBO J.*, **13**, 861–870.
- Frantz B and O'Neill EA. (1995). *Science*, **270**, 2017–2019.
- Fuchs SY, Chen A, Xiong Y, Pan Z-Q and Ronai Z. (1999). *Oncogene*, **18**, 2039–2046.
- Gabrilovich D, Ishida T, Oyama T, Ran S, Kravtsov V and Nadaf S. (1998). *Blood*, **92**, 4150–4166.
- Gehrt A, Erkel G, Anke T and Sterner O. (1998). *J. Antibiot.*, **51**, 455–463.
- Geng Z, Rong Y and Lau BH. (1997). *Free Rad. Biol. Med.*, **23**, 345–350.
- Gerbes AL, Vollmar AM, Kierner AK and Bilzer M. (1998). *Hepatology*, **28**, 1309–1317.
- Ghosh S, May MJ and Kopp EB. (1998). *Annu. Rev. Immunol.*, **16**, 225–260.
- Gilad E, Wong HR, Zingarelli B, Virag L, O'Connor M, Salzman AL and Szabo C. (1998). *FASEB J.*, **12**, 685–693.
- Grilli M, Pizzi M, Memo M and Spano P. (1996). *Science*, **274**, 1383–1385.
- Grimm S, Bauer MKA, Baeuerle PA and Schulze-Osthoff K. (1996). *J. Cell Biol.*, **134**, 13–23.
- Grisham MB, Palombella VJ, Elliott PJ, Conner EM, Brand S, Wong HL, Pien C, Mazzola LM, Destree A, Parent L and Adams J. (1999). *Methods Enzymol.*, **300**, 345–363.
- Grunberger D, Banerjee R, Eisinger K, Oltz EM, Efros L, Caldwell M, Estevez V and Nakanishi K. (1988). *Experientia*, **44**, 230–232.
- Harant H, Wolff B and Lindley IJD. (1998). *FEBS Lett.*, **436**, 329–334.
- Hehner SP, Heinrich M, Borks P, Vogt M, Ratter F, Lehmann V, Schulze-Osthoff K, Dröge W and Schmitz L. (1998). *J. Biol. Chem.*, **273**, 1288–1297.
- Hernandez-Presa MA, Bustos C, Ortego M, Tunon J, Ortega L and Egido J. (1998). *Am. J. Pathol.*, **153**, 1825–1837.
- Higuchi M, Singh S, Chan H and Aggarwal BB. (1995). *Blood*, **86**, 2248–2256.
- Hiramoto M, Shimizu N, Sugimoto K, Tang J, Kawakami Y, Ito M, Aizawa S, Tanaka H, Makino I and Handa H. (1998). *J. Immunol.*, **160**, 810–819.
- Hirano F, Tanaka H, Miura T, Hirano Y, Okamoto K, Makino Y and Makino I. (1998). *Immunopharmacology*, **39**, 31–38.
- Hirano M, Osada S-i, Aoki T, Hirai S-i, Hosaka M, Inoue J-i and Ohno S. (1996). *J. Biol. Chem.*, **271**, 13234–13238.
- Hsu H, Shu HB, Pan MG and Goeddel DV. (1996). *Cell*, **84**, 299–308.
- Imbert V, Rupec RA, Livolsi A, Pahl HL, Traenckner EB, Mueller-Dieckmann C, Farahifar D, Rossi B, Auberger P, Baeuerle PA and Peyron JF. (1996). *Cell*, **86**, 787–798.
- Iqbal M, Chatterjee S, Kauer JC, Das M, Messina P, Freed B, Biazzo W and Siman R. (1995). *J. Med. Chem.*, **38**, 2276–2277.
- Islam KN, Devaraj S and Kialal I. (1998). *Circulation*, **98**, 2255–2261.
- Israël N, Gougerot-Pocidal MA, Aillet F and Virelizier JL. (1992). *J. Immunol.*, **149**, 3386–3393.
- Iwasaki T, Uehara Y, Graves L, Rachie N and Bomsztyk K. (1992). *FEBS Lett.*, **298**, 240–244.
- Janssen YM and Sen CK. (1999). *Methods Enzymol.*, **300**, 363–374.
- Jin FY, Nathan C, Radzioch D and Ding A. (1997). *Cell*, **88**, 417–426.
- Jobin C, Hellerbrand C, Licato LL, Brenner DA and Sartor RB. (1998a). *Gut*, **42**, 779–787.
- Jobin C, Panja A, Hellerbrand C, Iimuro Y, DiDonato J, Brenner DA and Sartor RB. (1998b). *J. Immunol.*, **160**, 410–418.
- Kanegae Y, Tavares AT, Izpisua Belmonte JC and Verma IM. (1998). *Nature*, **392**, 611–614.
- Karin M. (1999). *Oncogene*, **18**, 6867–6874.
- Karin M and Delhase M. (1998). *Proc. Natl. Acad. Sci. USA*, **95**, 9067–9069.
- Katsuyama K, Shichiri M, Marumo F and Hirata Y. (1998). *Arterioscler. Thromb. Vasc. Biol.*, **18**, 1796–1802.
- Kawashima S, Hayashi M, Takii T, Kimura H, Zhang HL, Nagatsu A, Sakakibara J, Murata K, Oomoto Y and Onozaki K. (1998). *J. Interferon Cytokine Res.*, **18**, 423–428.
- Kazmi S, Plante R, Visconte V, Taylor G, Zhou L and Lau C. (1995). *J. Cell Biochem.*, **57**, 299–310.
- Kim CH, Kim JH, Moon SJ, Chung KC, Hsu CY, Seo JT and Ahn YS. (1999). *Biochem. Biophys. Res. Comm.*, **259**, 505–509.
- Kimura T, Sakaida I, Terai S, Matsumura Y, Uchida K and Okita K. (1997). *Biochem. Biophys. Res. Comm.*, **231**, 557–560.
- Kopp E and Ghosh S. (1994). *Science*, **265**, 956–959.
- Kotake Y, Sang H, Miyajima T and Wallis GL. (1998). *Biochim. Biophys. Acta*, **1448**, 77–84.
- Kumar A, Dhawan S and Aggarwal BB. (1998). *Oncogene*, **17**, 913–918.
- Kunz D, Walker G, Eberhardt W, Nitsch D and Pfeilschifter J. (1995). *Biochem. Biophys. Res. Comm.*, **216**, 438–446.
- Lauzurica P, Martinez-Martinez S, Marazuela M, Gomez de Arco P, Martinez C, Sanchez-Madrid F and Redondo JM. (1999). *Eur. J. Immunol.*, **26**, 1890–1900.
- Law RE, Stimmel JB, Damore MA, Carter C, Clarke S and Wall R. (1992). *Mol. Cell. Biol.*, **12**, 103–111.
- Lee FS, Hagler J, Chen ZJ and Maniatis T. (1997). *Cell*, **88**, 213–222.
- Lee FS, Peters RT, Dang LC and Maniatis T. (1998). *Proc. Natl. Acad. Sci. USA*, **95**, 9319–9324.
- Lentsch AB, Shanley TP, Sarma V and Ward PA. (1997). *J. Clin. Invest.*, **100**, 2443–2448.
- Le Page C, Sanceau J, Drapier JC and Wietzerbin J. (1998). *Biochem. Biophys. Res. Comm.*, **243**, 451–457.
- Li N and Karin M. (1998). *Proc. Natl. Acad. Sci. USA*, **95**, 13012–13017.
- Li N and Karin M. (1999). *FASEB J.*, **13**, 1137–1143.
- Lin YL and Lin JK. (1997). *Mol. Pharm.*, **52**, 465–472.
- Lin YZ, Yao SY, Veach RA, Torgerson TR and Hawiger J. (1995). *J. Biol. Chem.*, **270**, 14255–14258.
- Ling L, Cao Z and Goeddel DV. (1998). *Proc. Natl. Acad. Sci. USA*, **95**, 3792–3797.
- Mahboubi K, Young W and Ferreri NR. (1998). *J. Pharm. Exp. Ther.*, **285**, 862–868.

- Mahon TM and O'Neill LAJ. (1995). *J. Biol. Chem.*, **270**, 28557–28564.
- Malinin NL, Boldin MP, Kovalenko AV and Wallach D. (1997). *Nature*, **385**, 540–544.
- Manna SK and Aggarwal BB. (1998a). *J. Immunol.*, **161**, 2863–2872.
- Manna SK and Aggarwal BB. (1998b). *J. Immunol.*, **161**, 2873–2880.
- Manna SK and Aggarwal BB. (1998c). *J. Biol. Chem.*, **273**, 33333–33341.
- Manna SK and Aggarwal BB. (1999). *J. Immunol.*, **162**, 2095–2102.
- Manna SK, Gad YP, Mukhopadhyay A and Aggarwal BB. (1999a). *Biochem. Pharmacol.*, **57**, 763–774.
- Manna SK, Kuo MT and Aggarwal BB. (1999b). *Oncogene*, **18**, 4371–4382.
- Manna SK, Zhang HJ, Yan T, Oberley LW and Aggarwal BB. (1998). *J. Biol. Chem.*, **273**, 13245–13254.
- Marienfeld R, Neumann M, Chuvpilo S, Escher C, Kneitz B, Avots A, Schimpl A and Serfling E. (1997). *Eur. J. Immunol.*, **27**, 1601–1609.
- Matthews JR, Botting CH, Panico M, Morris HR and Hay RT. (1996). *Nucleic Acids Res.*, **24**, 2236–2242.
- McCaffrey PG, Kim PK, Valge-Archer VE, Sen R and Rao A. (1994). *Nucleic Acids Res.*, **22**, 2134–2142.
- Mercurio F, Zhu H, Murray BW, Shevchenko A, Bennett BL, Li J, Young DB, Barbosa M, Mann M, Manning A and Rao A. (1997). *Science*, **278**, 860–866.
- Meyer S, Kohler NG and Joly A. (1997). *FEBS Lett.*, **413**, 354–358.
- Mihm S, Ennen J, Pessara U, Kurth R and Dröge W. (1991). *AIDS*, **5**, 497–503.
- Mohan N, Sadeghi K, Reiter RJ and Meltz ML. (1995). *Biochem. Mol. Biol. Intl.*, **37**, 1063–1070.
- Musonada CA and Chipman JK. (1998). *Carcinogenesis*, **19**, 1583–1589.
- Natarajan K, Singh S, Burke Jr TR, Grunberger D and Aggarwal BB. (1996). *Proc. Natl. Acad. Sci. USA*, **93**, 9090–9095.
- Natarajan K, Manna SK, Chaturvedi MM and Aggarwal BB. (1998). *Arch. Biochem. Biophys.*, **352**, 59–70.
- Nemoto S, DiDonato JA and Lin A. (1998). *Mol. Cell Biol.*, **18**, 7336–7343.
- O'Connell MA, Bennett BL, Mercurio F, Manning AM and Mackman N. (1998). *J. Biol. Chem.*, **273**, 30410–30414.
- Okamoto S-I, Mukaida N, Yasumoto K, Rice N, Ishikawa Y, Horiguchi H, Murakami S and Matsushima K. (1994). *J. Biol. Chem.*, **269**, 8582–8589.
- Orth K, Palmer LE, Bao ZQ, Stewart S, Rudolph AE, Bliska JB and Dixon JE. (1999). *Science*, **285**, 1920–1923.
- Oyama T, Ran S, Ishida T, Nadaf S, Kerr L, Carbone DP and Gabrilovich DI. (1998). *J. Immunol.*, **160**, 1224–1232.
- Page S, Fischer C, Baumgartner B, Hass M, Kreusel U, Loidl G, Hayn M, Ziegler-Heitbrock HWL, Neumeier D and Brand K. (1999). *J. Biol. Chem.*, **274**, 11611–11618.
- Pahl HL, Krauss B, Schulze-Osthoff K, Decker T, Traenckner EB, Vogt M, Myers C, Warring P, Mülbacher A, Czernilofsky AP and Baeuerle PA. (1996). *J. Exp. Med.*, **183**, 1829–1840.
- Pahl HL. (1999). *Oncogene*, **18**, 6853–6866.
- Palayoor ST, Bump EA, Calderwood SK, Bartol S and Coleman CN. (1998). *Clin. Cancer Res.*, **4**, 763–771.
- Palombella VJ, Rando AL, Goldberg AL and Maniatis T. (1994). *Cell*, **78**, 773–786.
- Pierce JW, Read MA, Ding H, Luscinskas FW and Collins T. (1996). *J. Immunol.*, **156**, 3961–3969.
- Pierce JW, Schoenleber R, Jesmok G, Best J, Moore SA, Collins T and Gerritsen ME. (1997). *J. Biol. Chem.*, **272**, 21096–21103.
- Powell PP, Dixon LK and Parkhouse RME. (1996). *J. Virol.*, **70**, 8527–8533.
- Pyatt DW, Gruntmeir J, Stillman WS and Irons RD. (1998a). *Toxicology*, **128**, 83–90.
- Pyatt DW, Stillman WS and Irons RD. (1998b). *Toxicol. Appl. Pharmacol.*, **149**, 178–184.
- Qiu D, Zhao G, Aoki Y, Shi L, Uyei A, Nazarian S, Ng JCH and Kao PN. (1999). *J. Biol. Chem.*, **274**, 13443–13450.
- Ray A and Prefontaine KE. (1994). *Proc. Natl. Acad. Sci. USA*, **91**, 752–756.
- Reddy SA, Huang JH and Liao WS. (1997). *J. Biol. Chem.*, **272**, 29167–29173.
- Regnier CH, Song HY, Gao X, Goeddel DV, Cao Z and Rothe M. (1997). *Cell*, **90**, 373–383.
- Revilla Y, Callejo M, Rodriguez JM, Culebras E, Nogal ML, Salas ML, Vinuela E and Fresno M. (1998). *J. Biol. Chem.*, **273**, 5405–5411.
- Ritchie DM, Argentieri DC, Aparicio BL, Plante RK, Lau CY and Barbone AG. (1995). *Intl. J. Immunopharmacol.*, **17**, 805–812.
- Rodriguez MS, Thompson J, Hay RT and Dargemont C. (1999). *J. Biol. Chem.*, **274**, 9108–9115.
- Rossi A, Elia G and Santoro MG. (1997). *Proc. Natl. Acad. Sci. USA*, **94**, 746–750.
- Rossi A, Elia G and Santoro MG. (1998). *J. Biol. Chem.*, **273**, 16446–16452.
- Rothe M, Xiong J, Shu HB, Williamson K, Goddard A and Goeddel DV. (1996). *Proc. Natl. Acad. Sci. USA*, **93**, 8241–8246.
- Saliou C, Rihn B, Cillard J, Okamoto T and Packer L. (1998). *FEBS Lett.*, **440**, 8–12.
- Sappay C, Boelaert JR, Legrand-Poels S, Forceille C, Favier A and Piette J. (1995). *AIDS Res. Hum. Retroviruses*, **11**, 1049–1061.
- Scheinman RI, Cogswell PC, Lofquist AK and Baldwin Jr AS. (1995). *Science*, **270**, 283–286.
- Schesser K, Spiik AK, Dukuzumuremyi JM, Neurath MF, Pettersson S and Wolf-Watz H. (1998). *Mol. Microbiol.*, **28**, 1067–1079.
- Schreck R, Albermann K and Baeuerle PA. (1992b). *Free Rad. Res. Comm.*, **17**, 221–237.
- Schreck R, Meier B, Männel DN, Dröge W and Baeuerle PA. (1992a). *J. Exp. Med.*, **175**, 1181–1194.
- Schreck R, Rieber P and Baeuerle PA. (1991). *EMBO J.*, **10**, 2247–2258.
- Schulze-Osthoff K, Beyaert R, Vandervoorde V, Haegeman G and Fiers W. (1993). *EMBO J.*, **12**, 3095–3104.
- Sen CK, Traber K and Packer L. (1996a). *Biochem. Biophys. Res. Comm.*, **218**, 148–153.
- Sen CK, Roy S and Packer L. (1996b). *FEBS Lett.*, **85**, 58–62.
- Sen CK, Tirosch O, Roy S, Kobayashi MS and Packer L. (1998). *Biochem. Biophys. Res. Comm.*, **247**, 223–228.
- Sen J, Venkataraman L, Shinkai Y, Pierce JW, Alt FW, Burakoff SJ and Sen R. (1995). *J. Immunol.*, **154**, 3213–3221.
- Serfling E, Avots A and Neumann M. (1995). *Biochim. Biophys. Acta*, **1263**, 181–200.
- Shoji S, Furuishi K, Ogata A, Yamataka K, Tachibana K, Mukai R, Uda A, Harano K, Matsushita S and Misumi S. (1998). *Biochem. Biophys. Res. Comm.*, **249**, 745–753.
- Shrivastava A, Manna SK, Ray R and Aggarwal BB. (1998). *J. Virol.*, **72**, 9722–9728.
- Shumilla JA, Wetterhahn KE and Barchowski A. (1998). *Arch. Biochem. Biophys.*, **349**, 356–362.
- Siebenlist U, Franzo G and Brown K. (1994). *Annu. Rev. Cell Biol.*, **10**, 405–455.
- Simpson CS and Morris BJ. (1999). *J. Neurochem.*, **73**, 353–361.
- Singh S and Aggarwal BB. (1995a). *J. Biol. Chem.*, **270**, 10631–10639.
- Singh S and Aggarwal BB. (1995b). *J. Biol. Chem.*, **270**, 24995–25000.

- Singh S, Darnay BG and Aggarwal BB. (1996a). *J. Biol. Chem.*, **271**, 31049–31054.
- Singh S, Natarajan K and Aggarwal BB. (1996b). *J. Immunol.*, **10**, 4412–4420.
- Sizemore N, Leung S and Stark GR. (1999). *Mol. Cell Biol.*, **19**, 4798–4805.
- Song HY, Regnier CH, Kirschning CJ, Goeddel DV and Rothe M. (1997). *Proc. Natl. Acad. Sci. USA*, **94**, 9792–9796.
- Spiecker M and Liao JK. (1999). *Methods Enzymol.*, **300**, 375–389.
- Staal FJT, Roederer M, Raju PA, Anderson MT, Ela SW and Herzenberg LA. (1993). *AIDS Res. Hum. Retroviruses*, **9**, 299–305.
- Sueoka E, Sueoka N, Kai Y, Okabe S, Suganuma M, Kanematsu K, Yamamoto T and Fujiki H. (1998). *Biochem. Biophys. Res. Comm.*, **252**, 566–570.
- Sugano N, Shimada K, Ito K and Murai S. (1998). *Biochem. Biophys. Res. Comm.*, **252**, 25–28.
- Sun WH, Keller ET, Stebler BS and Ershler WB. (1998). *Biochem. Biophys. Res. Comm.*, **244**, 691–695.
- Suzuki YJ, Aggarwal BB and Packer L. (1992). *Biochem. Biophys. Res. Comm.*, **189**, 1709–1715.
- Suzuki YJ and Packer L. (1993a). *Biochem. Biophys. Res. Comm.*, **193**, 277–283.
- Suzuki YJ and Packer L. (1993b). *Biochem. Mol. Biol. Intl.*, **31**, 693–700.
- Suzuki YJ and Packer L. (1994). *Biochem. Mol. Biol. Intl.*, **32**, 299–305.
- Suzuki YJ, Mizuno M, Tritschler HJ and Packer L. (1995). *Biochem. Mol. Biol. Intl.*, **36**, 241–246.
- Tepper MA, Nadler SG, Esselstyn JM and Sterbenz KG. (1995). *J. Immunol.*, **155**, 2427–2436.
- Toledano MB and Leonard WJ. (1991). *Proc. Natl. Acad. Sci. USA*, **88**, 4328–4332.
- Trepicchio WL and Dorner AJ. (1998). *Ann. NY Acad. Sci.*, **856**, 12–21.
- Umansky V, Shatrov VA, Lehmann V and Schirmacher V. (1996). *Intl. Immunol.*, **8**, 491–498.
- van Antwerp DJ, Martin SJ, Kafri T, Green DR and Verma IM. (1996). *Science*, **274**, 787–789.
- van Hogerlinden M, Rozell BL, Åhrlund-Richter L and Toftgård R. (1999). *Cancer Res.*, **59**, 3299–3303.
- Venkataraman L, Burakoff SJ and Sen R. (1995). *J. Exp. Med.*, **181**, 1091–1099.
- Venkataraman L, Wang W and Sen R. (1996). *J. Immunol.*, **157**, 1149–1155.
- Wahl C, Liptay S, Adler G and Schmid RM. (1998). *J. Clin. Invest.*, **101**, 1163–1174.
- Wang C-Y, Mayo MW and Baldwin Jr AS. (1996). *Science*, **274**, 784–787.
- Wang C-Y, Cusack Jr JC, Liu R and Baldwin Jr AS. (1999). *Nature Med.*, **5**, 412–417.
- Wang JY, Suo JS, Li H, Liu S-L and Zern MA. (1998). *Liver*, **18**, 180–185.
- Wang W, Tam WF, Hughes CC, Rath S and Sen R. (1997). *Immunity*, **6**, 165–174.
- Wechsler AS, Gordon MC, Dendorfer U and LeClair KP. (1994). *J. Immunol.*, **153**, 2515–2523.
- Weissmann G. (1991). *Hosp. Pract.*, **26**, 60–76.
- Whiteside ST and Israël A. (1997). *Sem. Cancer Biol.*, **8**, 75–82.
- Woronicz JD, Gao X, Cao Z, Rothe M and Goeddel DV. (1997). *Science*, **278**, 866–869.
- Yamamoto Y, Yin M-J, Lin K-M and Gaynor RB. (1999). *J. Biol. Chem.*, **274**, 27307–27314.
- Yamaoka S, Courtois G, Bessia C, Whiteside ST, Weil R, Agou F, Kirk HE, Kay RJ and Israël A. (1998). *Cell*, **93**, 1231–1240.
- Yang F, de Villiers WJS, McClain CJ and Varilek GW. (1998). *J. Nutr.*, **128**, 2334–2340.
- Yang J, Merin JP, Nakano T, Kato T, Kitade Y and Okamoto T. (1995). *FEBS Lett.*, **361**, 89–96.
- Yaron A, Gonen H, Alkalay I, Hatzubai A, Jung S, Beyth S, Mercurio F, Manning AM, Ciechanover A and Ben-Neriah Y. (1997). *EMBO J.*, **16**, 6486–6494.
- Yeh WC, Shahinian A, Speiser D, Kraunus J, Billia F, Wakeham A, de la Pompa JL, Ferrick D, Hum B, Iscove N, Ohashi P, Rothe M, Goeddel DV and Mak TW. (1997). *Immunity*, **7**, 715–725.
- Yin M-J, Yamamoto Y and Gaynor RB. (1998). *Nature*, **396**, 77–80.
- Yoshida A, Yoshida S, Ishibashi Y, Kuwano M and Inomata H. (1999). *Invest. Ophthalmol. Vis. Sci.*, **40**, 1624–1629.
- Zandi E, Rothwarf DM, Delhase M, Hayakawa M and Karin M. (1997). *Cell*, **91**, 243–252.