

Molecular Control of Immune/Inflammatory Responses: Interactions Between Nuclear Factor- κ B and Steroid Receptor-Signaling Pathways

LORRAINE I. MCKAY AND JOHN A. CIDLOWSKI

Laboratory of Signal Transduction, Molecular Endocrinology Group, National Institute of Environmental Health Sciences, National Institutes of Health, Research Triangle Park, North Carolina 27709

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I. Introduction

THE mammalian immune and inflammatory responses are complex physiological processes that are critically important to the homeostasis and ultimate survival of an organism. Their coordinate regulation must be assured to allow appropriate and timely immune reaction without an overreaction that might cause damage to the host. Two cellular signaling pathways that have been identified as important regulators of immunity and inflammation are the

nuclear factor (NF)- κ B and glucocorticoid-mediated signal transduction cascades.

Glucocorticoid receptor- α (GR) and NF- κ B are inducible transcription factors with diametrically opposed functions in the regulation of immune and inflammatory responses. NF- κ B is known to mediate the transcriptional activation of a variety of cytokines and cytokine-induced genes involved in immunity. GR, long known to function as a suppressor of immunity and inflammation, inhibits the expression of many of the same cytokines and cytokine-induced genes that are activated by NF- κ B. However, because these genes have no identifiable glucocorticoid responsive elements (GRE) either within their promoters or their intragenic regions, the mechanism by which glucocorticoids repress these genes was unclear.

Recent research indicates that GR and NF- κ B physically interact (1–3) and function as mutual transcriptional antagonists (1, 4). These findings have rekindled interest in, and encouraged re-examination of, these interactions as the basis for mutual functional antagonism of GR and NF- κ B. While physiological processes in a variety of tissues and organ systems are regulated by glucocorticoids and are likely to be modulated by GR interactions with the ubiquitous NF- κ B, the immune/inflammatory response has been the primary focus of current research on NF- κ B/GR antagonism. Since both these transcription factors are known to be potent regulators of the immune system, elucidation of the mechanisms by which GR and NF- κ B negatively interact not only provides the basis for understanding their role in the precise control of normal immune response, but also opens up the possibility of novel therapeutic intervention in immune pathology.

The purpose of this review is to describe our current knowledge of NF- κ B and GR-signaling pathways, including overviews of the NF- κ B and steroid hormone receptor families of transcription factors and their regulatory proteins, their general mechanisms of action, the cell types in which they function, and their regulated genes. Special consideration will then be given to the newest findings concerning the interactions between these two transcription factors, and the physiological significance of these findings in terms of immunity and inflammation. Finally, emerging data concern-

Address reprint requests to: John A. Cidlowski, Ph.D., Laboratory of Signal Transduction, National Institute of Environmental Health Sciences, P.O. Box 12233, MD E2-O2, 111 TW Alexander Drive, Research Triangle Park, North Carolina 27709 USA. E-mail: Cidlowski@NIEHS.NIH.gov

ing interactions between NF- κ B and other steroid hormone receptors will be considered.

II. Nuclear Factor- κ B (NF- κ B)

A. Introduction

NF- κ B was identified more than a decade ago by Sen and Baltimore (5) as an enhancer-binding protein controlling Ig κ -light chain gene expression in B cells. This seminal paper identified the NF- κ B protein as an activity in electrophoretic mobility shift assays that specifically retarded the migration of κ -light chain enhancer DNA containing the 10-mer sequence 5'-GGACTTCC-3'. Research that followed this initial finding indicated that this κ -enhancer binding activity was only found in nuclear extracts from B cells that were at an appropriate stage to express Ig κ -light chain, and showed that NF- κ B was an essential factor for the function of the κ -light chain enhancer (6, 7). Because of this apparent B cell specificity, NF- κ B was considered to be a tissue-restricted transcription factor. However NF- κ B was later found to be expressed not only in B cells, but in other immune cells as well. In non-B cell lymphocytes, NF- κ B was found to be an inactive protein sequestered in the cytoplasm, which could be activated with phorbol ester and lipopolysaccharide, rather than as the constitutively active nuclear protein seen in B cells (8–10). Of particular interest was the finding that, in T cells with latent human immunodeficiency virus (HIV) infection, NF- κ B could be activated by a variety of compounds that cause lymphokine secretion. Once activated, NF- κ B enhanced the expression of HIV in these cells (10). This exciting finding led to a proliferation of research in the fledgling field of NF- κ B research.

Today, NF- κ B is recognized as a ubiquitously expressed transcription factor that can be activated in a wide variety of cell types. Despite the discovery that NF- κ B enjoys a wide pattern of cellular expression, the particular importance of NF- κ B to cells of the immune system remains. In immune cells, NF- κ B has now been shown to positively regulate the expression of a wide variety of genes involved in mammalian immune and inflammatory responses, including cytokines, cell adhesion molecules, complement factors, antiapoptotic factors, and immunoreceptors (for a detailed list, refer to Table 1).

B. NF- κ B is a dimeric transcription factor

NF- κ B functions as a dimeric DNA-binding protein that comprises subunits from a family of related proteins called the Rel family of transcriptional activators. The general mechanism of NF- κ B signaling by the prototypical p65/p50 heterodimer, with phosphorylation by the catalytic subunit of protein kinase A (PKAc), is depicted in Fig. 1 and is discussed in detail in a later section of this review. A schematic overview of Rel family members is depicted in Fig. 2. To date, the Rel family members identified include the mammalian proteins p65 (Rel A), Rel B, c-Rel, p50/p105 (NF- κ B1), p100/p52 (NF- κ B2), and the *Drosophila melanogaster* proteins Dorsal (a dorsoventral pattern formation gene) and Dif (which mediates immune response in *Drosophila* larvae) (11–

22). As shown in the accompanying diagram, p50 and p52 are proteolytic cleavage products of precursor proteins that possess both a Rel region and an I κ B-like region (described below). There is also evidence of a Rel-like protein in *Saccharomyces cerevisiae* that is involved in cell growth (23). Recently, a more distantly related and less well characterized *Drosophila* transcriptional activator protein was identified. Termed Relish, this protein possesses both Rel-like and I κ B-like (ankyrin repeats; see below) domains and is believed to regulate embryogenesis and some immune responses (24). These Rel proteins possess a highly conserved region of approximately 300 amino acids known as the Rel homology domain. Contained within this Rel homology domain are the DNA recognition/binding, dimerization, and nuclear localization functions of NF- κ B (25–28). A more detailed description of the domain structure of p65 is found in a subsequent section of the text.

The various members of the Rel family can homodimerize or heterodimerize with other Rel proteins to form DNA binding-competent NF- κ B factors with different sequence specificities (22, 28–32). While not all combinations of NF- κ B dimers have been shown to form, and not all dimers that can form have been shown to have physiological relevance, several transcriptionally active NF- κ B dimers have been identified, including relB/p50, Rel B/p52, p65/p50, p65/p65, p65/c-Rel, and possibly p50/p50. The relevance of p50 homodimer's ability to induce transcription in yeast and cell-free systems compared with its function in mammalian cells remains a matter of dispute (see Refs. 33–37 for varying viewpoints).

Since p50 and p52 lack the transactivation domain found in the other Rel family members, it is not clear whether homodimers of these proteins have any transcriptional activity in native environments. One study (38) indicates that p52 homodimers can directly transactivate when associated with the I κ B-like protein *bcl-3*. In general, however, it is believed that an NF- κ B dimer must possess at least one Rel transactivation domain to activate transcription *in vivo*. The most widely accepted function for p50 and p52 homodimers is as transcriptional repressors that block NF- κ B sites in the DNA from transcriptionally active NF- κ B dimers (39). Evidence for a repressive function of p50 homodimers was found in human T cells, where p50 homodimers constitutively expressed in the nucleus bind DNA and repress NF- κ B-dependent transcription. This repression was overcome by overexpression of *bcl-3*, which has been shown to prevent binding of p50 homodimers to NF- κ B sites *in vitro* and to activate NF- κ B-mediated transcription in cultured cells (35, 40). Whether this observed *bcl-3*-mediated increase in transcription is entirely due to its repressive effect on p50 homodimers is unclear, however, because *bcl-3* can function as a transactivator at NF- κ B sites when it trimerizes with otherwise transcriptionally inactive p52 homodimers (38).

The composition of NF- κ B dimers determines sequence specificity by combining different DNA recognition loops in the Rel homology domains of different subunits. For example, p65 homodimers preferentially bind DNA enhancer motifs that are not well recognized by p65/p50 heterodimers or p50/p50 homodimers (31). Such variety in dimerization may contribute to the cell type specificity of NF- κ B response. For

TABLE 1. Genes transcriptionally regulated by NF- κ B

Type of molecule	Gene	Relevant cell types
Growth factors	Granulocyte/macrophage colony stimulating factor (G/M-CSF)	Monocytes/macrophages
	Macrophage CSF Granulocyte CSF	Myeloid cells
Cell adhesion molecules	MAd-CAM-1	Mucosal cells
	ELAM-1	
	ICAM-1	Brain, endothelial cells
	VCAM	
	E-Selectin P-Selectin	Endothelial cells, megakaryocytes
Cytokines/chemokines	IL-1	B cells
	IL-2	T cells
	IL-6	B cells, T cells, osteoclasts
	IL-8	
	TNF- α	B cells, T cells, monocytes, macrophages, etc.
	β -Interferon	
	TNF- β (lymphotoxin)	
	γ -Interferon	
	RANTES	Lung epithelium
	MCP-1/JE Gro - α , - β , - γ	Monocytes/macrophages
Acute phase proteins	Angiotensinogen	Hepatocytes
	Serum amyloid A precursor protein	
	Complement factors (B and C4)	
Transcriptional regulators	p53	Immune cells
	c-rel, v-rel	
	I κ B α	
	NF κ B p105	
	c-myc	B cells, Burkitt's lymphoma
Immunoreceptors	Tissue factor-1	
	β -2 Microglobulin	
	T cell receptors (α and β)	T cells
	Major histocompatibility complex class I and II proteins	Multiple cell types
Viruses	HIV-1	
	CMV	
	SV-40	
	Adenovirus	
Antiapoptotic	TRAF-1	
	TRAF-2	Immune cells and cultured fibrosarcoma cells
	c-IAP1	
	c-IAP2	
Other	A-20	B cells
	Vimentin	Multiple cell types
	Proteasomal LMP2	
	iNOS	
	COX2	
	TAP1	

Table was compiled from Refs. 22, 28, 91, 98, and 200–215.

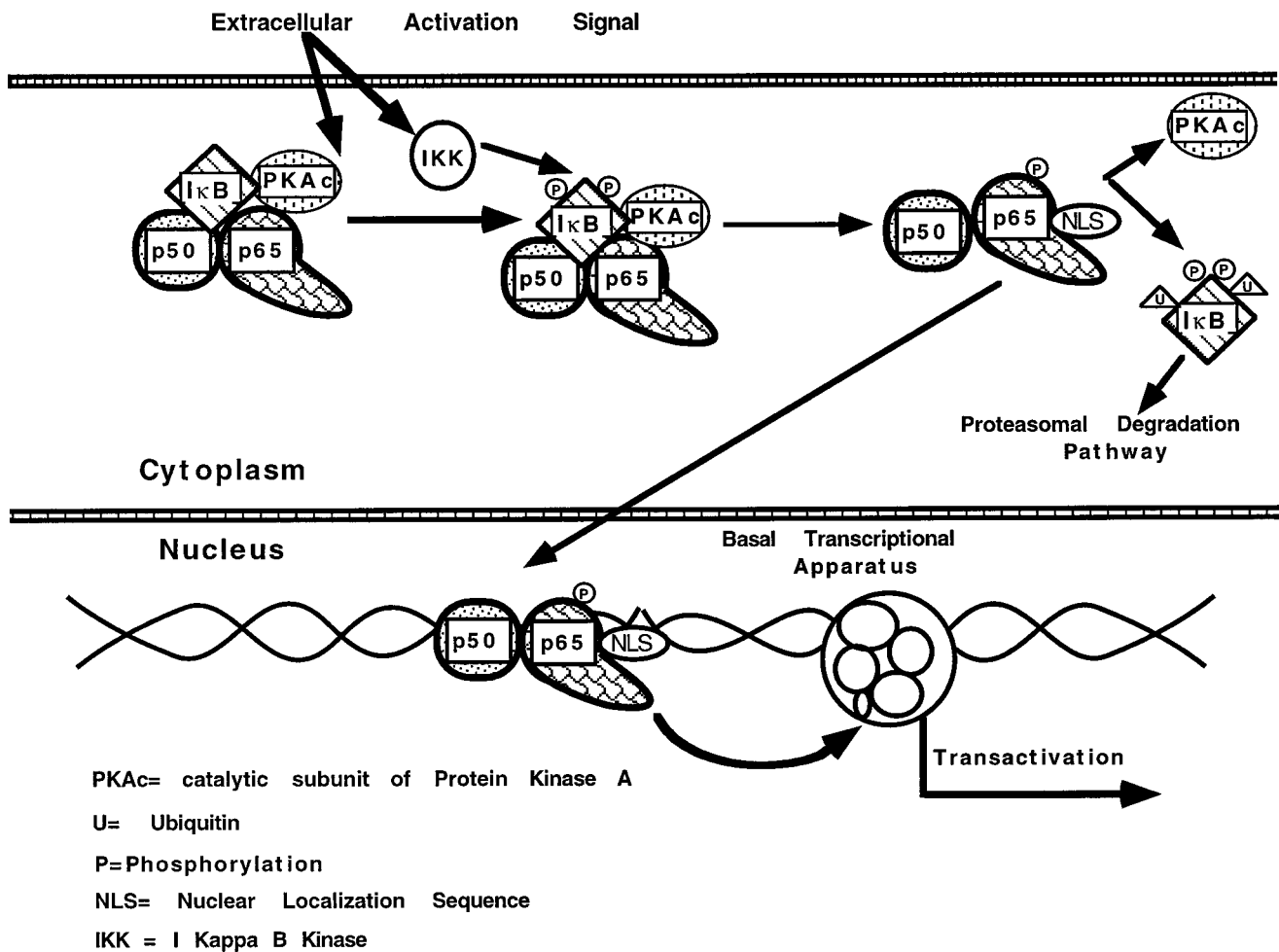


FIG. 1. The general mechanism of NF- κ B activation. Unactivated NF- κ B heterodimers are retained in the cytoplasm by the inhibitory NF- κ B subunit I κ B. Cytoplasmic NF- κ B is associated with the catalytic subunit of protein kinase A (PKAc). In response to an activating signal, I κ B kinase (IKK) phosphorylates I κ B, targeting it for ubiquitination and subsequent degradation via the proteasome, while PKAc phosphorylates the p65 subunit of NF- κ B. This activation process exposes a nuclear localization signal on p65, allowing the activated NF- κ B heterodimer to move into the cell nucleus where it interacts with κ B-responsive elements in the chromatin and modulates gene transcription.

example, cell type differences in NF- κ B activity might depend on which subunits of NF- κ B are expressed (and in what ratios) and on the NF- κ B activating signals to which these different cells respond. Dimer composition may also affect interactions with inhibitory/regulatory proteins such as the various members of the I κ B family of proteins (22, 28, 32, 35, 41–43). The ability to modulate gene expression in a cell type-specific or temporally regulated manner by altering the expression patterns of various Rel subunits affords NF- κ B-mediated signal transduction the potential for both great flexibility and tight control.

Several gene knockout mice that are deficient in one or more specific NF- κ B subunits have been generated. The phenotypes of these transgenic animals vary widely and will be discussed in detail in a later section of this review. When taken as a whole, the information gleaned from NF- κ B knockouts solidifies the importance of NF- κ B to immune function, inflammatory response, and development.

p65/p50: the "Classical" NF- κ B. The prototypical and most thoroughly studied NF- κ B dimer is the p65/p50 het-

erodimer. When these two subunits are coexpressed at comparable levels in the cell, their affinity for each other is higher than the affinity of either homodimer, and therefore the heterodimer is preferentially formed (22). This heterodimer has a high affinity for the consensus NF- κ B DNA sequence 5'-GGGRNNYYCC-3' (22, 28) and is generally considered to be the predominant, inducible (*i.e.*, activatable by extracellular signals, nonconstitutive) form of NF- κ B in most cells. For this reason, throughout the remainder of this text, the term NF- κ B will refer to the "classical" NF- κ B p65/p50 heterodimer unless otherwise specified.

As the transcriptionally active subunit of the prototypical NF- κ B heterodimer, the structure of the p65 (Rel A) polypeptide has been extensively studied. The transactivation function of p65 has been mapped to a 120-amino acid region of the C terminus, which contains two distinct and independent transactivation domains (27). The first domain comprises 30 amino acids at the extreme C terminus and is known as TA₁, whereas TA₂ is located within the adjacent 90 amino acids of the C-terminal region of p65. These transactivation domains

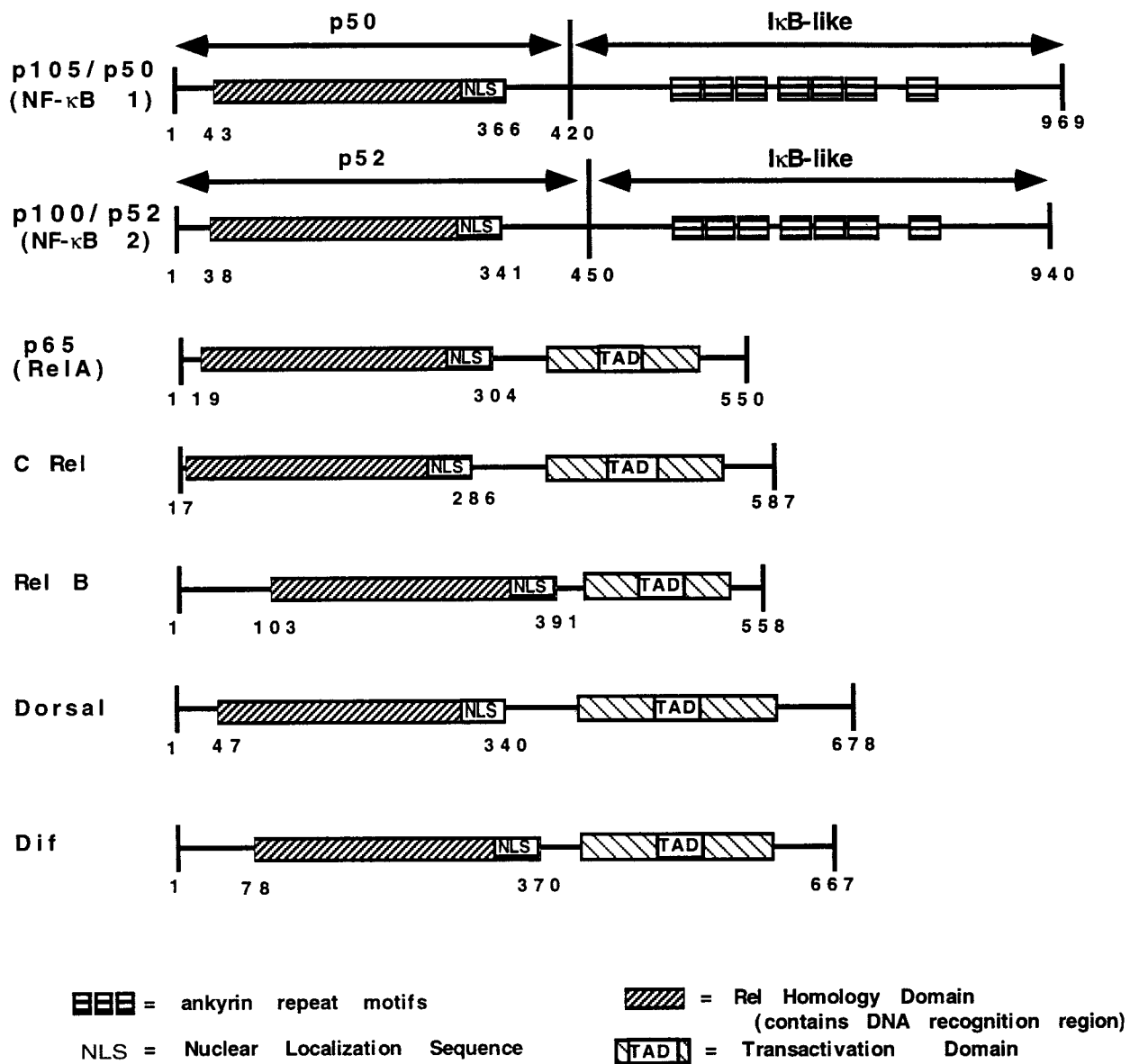


FIG. 2. Schematic overview of the Rel family of transcriptional activators. All family members contain a highly conserved Rel homology domain of approximately 300 amino acids, which contains sequences required for dimerization, nuclear localization, and DNA recognition. Dorsal and Dif are *Drosophila* gene products, while all others identified are mammalian. The p50 and p52 transcriptional activators are proteolytically cleaved from the p105 and p100 precursor molecules, respectively. They lack the transactivation domain that is present in all other Rel proteins.

share a common sequence motif that is induced to form an α -helical conformation when bound to target molecules (27, 44). Phorbol ester-induced phosphorylation of the TA₂ domain between amino acids 442 and 470 has been shown to enhance the transcriptional activity of p65 (44). Recent crystallographic data (45) have also revealed the structure of DNA-bound p65/p50. These data demonstrate that the subunits dimerize via β -sheet sandwich structures in the C-terminal dimerization domains, and contacts with the DNA consensus sequence are made using loops from the ends of the N- and C-termini. Beg *et al.* (46) have shown that the nuclear localization sequence located in the middle of the p65 polypeptide is the target for interaction with the inhibitory subunit I κ B.

C. The regulatory subunit I κ B is an inhibitor of NF- κ B

Not long after the initial identification of NF- κ B, Baeuerle and Baltimore (47) identified an inhibitory protein of 60–70 kDa that specifically associates with NF- κ B dimers, forming a trimer that cannot bind DNA and is retained in the cell cytoplasm. They termed this protein I κ B, for inhibitor of NF- κ B. More recently, there have been multiple I κ B proteins identified with different specificities for NF- κ B dimers and different cell type distributions. This family of I κ Bs now includes I κ B α , I κ B β , I κ B γ , I κ B-R, Bcl-3, p50/p105(C-terminus), p100/p52(C-terminus), and the *Drosophila melanogaster* proteins Cactus and (probably) Relish (14–17, 21, 24, 28, 48–57). The I κ B family of proteins is outlined schematically

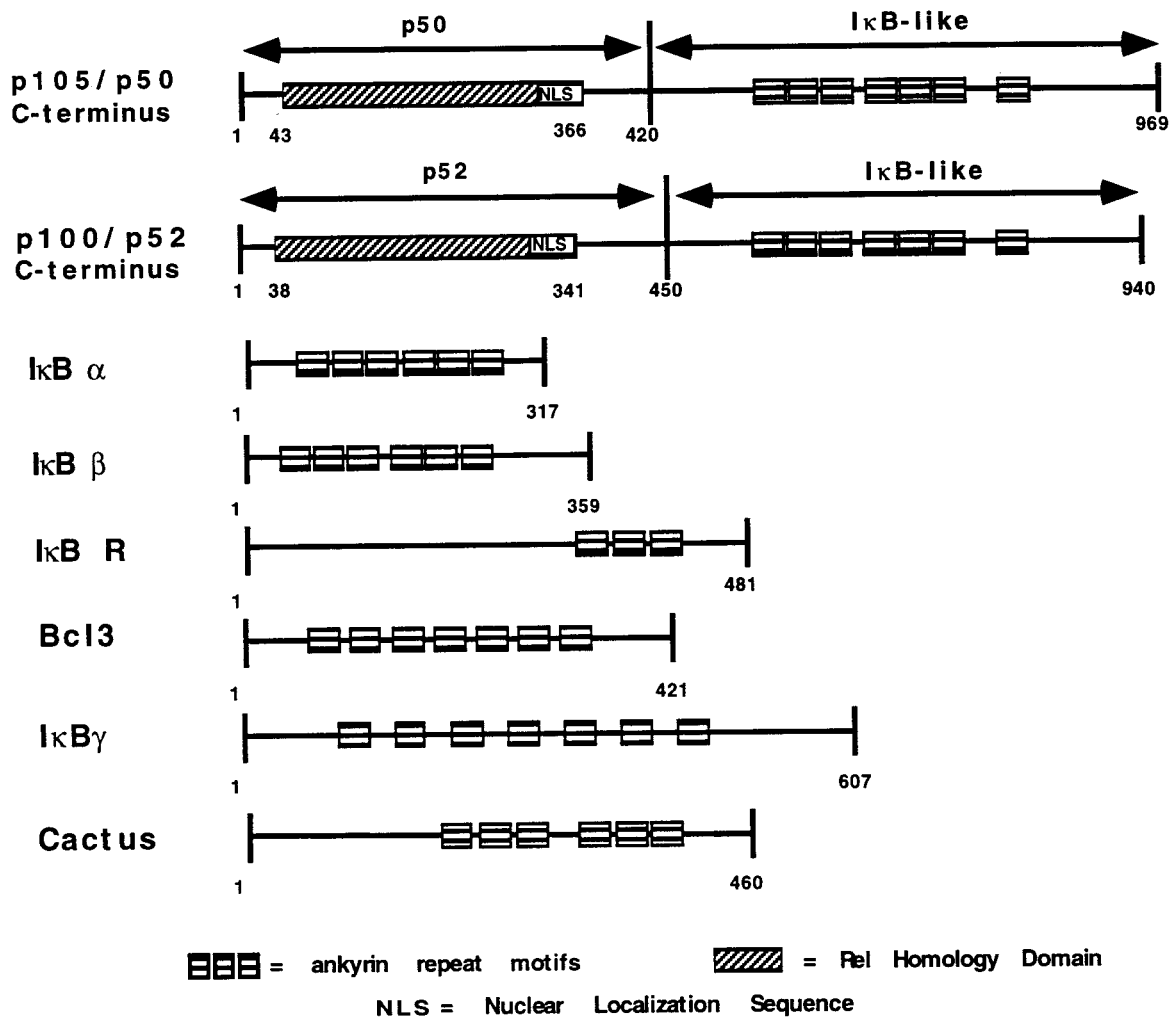


FIG. 3. The $\text{I}\kappa\text{B}$ family of regulatory proteins. All family members contain three to seven ankyrin repeat domains, which mediate associations with $\text{NF-}\kappa\text{B}$. These proteins also contain a C-terminal PEST sequence and a variable acidic region in the C terminus. p105 and p100 are precursor proteins that have $\text{I}\kappa\text{B}$ -like structure and activity in the C-terminal portion of the molecule.

in Fig. 3. There is also evidence that the yeast *Saccharomyces cerevisiae* expresses an $\text{I}\kappa\text{B}$ -like protein (23). $\text{I}\kappa\text{B}\alpha$ and $\text{I}\kappa\text{B}\beta$ both preferentially interact with dimers containing p65, but they are responsive to different signaling pathways in the cell and may be differentially expressed and employed in different cell types (28, 58). The p100/p52 and p50/p105 molecules contain both an $\text{NF-}\kappa\text{B}$ -like and an $\text{I}\kappa\text{B}$ -like region. The $\text{I}\kappa\text{B}$ region can be cleaved from the $\text{NF-}\kappa\text{B}$ region to yield two functional and distinct molecules or can act intramolecularly to inhibit the function of the $\text{NF-}\kappa\text{B}$ portion of the intact molecule.

The common features of all $\text{I}\kappa\text{B}$ s identified thus far are the presence of multiple ankyrin repeat motifs, involved in the protein-protein interaction between $\text{NF-}\kappa\text{B}$ and $\text{I}\kappa\text{B}$, an acidic region in the C terminus of the proteins, and a C-terminal PEST (pro-glu/asp-ser-thr) sequence (21, 51). Whereas the acidic region of the $\text{I}\kappa\text{B}$ s is believed to be involved in interaction with the $\text{NF-}\kappa\text{B}$ DNA-binding/nuclear localization region, and the PEST sequence may be involved in regulating $\text{I}\kappa\text{B}$ degradation, the functions of these two regions have not been conclusively demonstrated and remain a matter of de-

bate. (Although beyond the scope of this review, evidence supporting both sides of this question is discussed in Ref. 21).

D. Activation and function of $\text{NF-}\kappa\text{B}$

Constitutive $\text{NF-}\kappa\text{B}$ activity in B cells is largely attributable to the p50-cREL heterodimer (28, 59, 60). However, in most other cell types, $\text{NF-}\kappa\text{B}$ activity is largely inducible, and the prototype p65/p50 $\text{NF-}\kappa\text{B}$ is retained in the cytoplasm of an unstimulated cell by its association with $\text{I}\kappa\text{B}\alpha$. When an extracellular stimulus, such as a cytokine (e.g., tumor necrosis factor- α), viral protein, lipopolysaccharide, or an oxidative stressor activates the $\text{NF-}\kappa\text{B}$ -signaling pathway, $\text{I}\kappa\text{B}\alpha$ is phosphorylated at serines 32 and 36 (61). Phosphorylated $\text{I}\kappa\text{B}\alpha$ is a target for ubiquitination at lysines 21 and 22 (21, 62, 63), which leads to rapid removal of $\text{I}\kappa\text{B}\alpha$ via the proteosomal degradation pathway and, consequently, unmasking of the p65 nuclear localization sequence and movement of $\text{NF-}\kappa\text{B}$ to the nucleus of the cell (64, 65). [There are two different but related ser/thr kinases, identified by several groups and now termed IKK-1 and IKK-2, that are generally accepted to be

responsible for the inducible phosphorylation of I κ B (66–69). A very recent study indicates that these IKKs have a greater phosphorylation activity on I κ B when it is bound to NF- κ B, thus explaining the ability of active IKK and free I κ B to coexist within a cell and regulate NF- κ B (70). Regulation of the activity of these kinases provides an indirect mechanism for the regulation of NF- κ B activation.]

Although not yet completely understood, the activation of NF- κ B involves not only the dissociation of I κ B and translocation to the nucleus, but also phosphorylation of the p65 subunit by PKAc. This PKAc is associated with the NF- κ B/I κ B heterotrimer in the cytoplasm and is believed to be held inactive by the association with I κ B. Once I κ B dissociates, the PKAc becomes active, phosphorylates serine 276 of p65 (at a consensus PKA site in the Rel domain of the protein), and dramatically increases the transcriptional activity of NF- κ B (71). Recent studies show that this PKA-mediated phosphorylation of p65 is required for recruitment of CREB-binding protein and the closely related p300 (CBP/p300), a transcriptional coactivator discussed below, by NF- κ B p65 (72). There is evidence that other protein kinases may also be important regulatory proteins for p65. For example, casein kinase II has been shown to mediate the interleukin-1 (IL-1)-stimulated phosphorylation of p65 in fibroblasts and hepatoma cells (73). It is possible that the phosphorylation required for p65 activation is mediated by various kinases in a cell type-specific manner.

The classical p65/p50 NF- κ B heterodimer is a major inducer of inflammatory genes, and there are a large number of proinflammatory, extracellular signals that can activate NF- κ B. These stimuli include viruses [such as herpes simplex virus and adenovirus] and viral proteins; bacterial products like lipopolysaccharide; inflammatory cytokines like TNF α , IL-1, and IL-2; and a variety of DNA-damaging agents and oxidative stressors (reviewed in Ref. 22). Once activated, the nuclear NF- κ B binds to cognate NF- κ B sites in the chromatin and modulates gene expression. A variety of NF- κ B-responsive genes involved in immune response and inflammation due to these extracellular proinflammatory signals are induced by activated NF- κ B. New synthesis of I κ B causes retention of NF- κ B in the cytoplasm and attenuation of NF- κ B-mediated transcriptional activation and provides a feedback mechanism for modulating the extent and duration of inflammatory responses by the cell.

Genes that are known to be regulated by NF- κ B are listed in Table 1. In addition to the well studied role of NF- κ B in the immune system (including lymphocyte development, inflammatory response, and host defense mechanisms), more recent work has also implicated NF- κ B as an important regulator of apoptosis and embryonic development (74).

E. The transcription factor NF- κ B interacts with multiple transcription factors and transcriptional cofactors

With the advent of yeast two-hybrid screening techniques, a variety of enhancer binding transcription factors have been found to interact with a variety of nuclear proteins. Many of these interacting nuclear proteins have been identified as transcriptional cofactors, which have the ability to either enhance or repress the transactivation function of transcrip-

tion factors (for example, see Refs. 75–77). As knowledge in the field of transcriptional regulation expands, a consensus is forming that transcription factors do not usually function alone as independent activators of transcription. Rather than the classical view that these proteins bind DNA and then directly contact the basal transcriptional machinery, the current understanding is that DNA-binding transcription factors often interact simultaneously with multiple nuclear proteins that are either transcription factors themselves or accessory proteins. The resulting large transcription-regulatory complexes interact with the basal transcription apparatus to modulate gene expression. The combination of transcription-regulatory proteins “piled up” on the promoter of a gene allows for cell type, developmental, promoter, and stimulus-driven specificities in gene expression regulated by a transcription factor. Examples of such promoter complexes are depicted in reports by Merika *et al.* (75), which deals with the “enhanceosome” complex, and Shibata *et al.* (78), which examines complexes containing members of the nuclear receptor superfamily.

As the list of proteins that interact with NF- κ B and alter its transactivation function grows, it is becoming clear that NF- κ B is no exception to this general rule of transcription-regulatory complex formation. The complexity and potential flexibility of NF- κ B effects in the cell are suggested both by the broad range of NF- κ B-interacting proteins that have been identified and by the fact that some of these proteins appear to have context-dependent effects, acting antagonistically with NF- κ B in some cases and synergistically in others. The p65 subunit of NF- κ B has been shown to directly interact *in vitro* with the general transcription factors TFIIB and TATA-binding protein (TBP) (79). A functional interaction between TBP and p65 (as well as c-Rel), which activates NF- κ B-dependent transcription, has been shown in cultured insect and mammalian cells (79–82). These data reveal a potentially important mechanism for NF- κ B-mediated regulation of the basal transcriptional apparatus.

As previously mentioned, *Bcl-3* was originally identified to be an inhibitory subunit of NF- κ B and is now known to function as a specific transcriptional coactivator of the p52 and p50 members of the Rel family (38). HMG-1 (high mobility group-1) coactivates NF- κ B-mediated transcription, and SRC-1 specifically coactivates NF- κ B p50 (83, 84). Members of the CCAAT-enhancer binding protein (C/EBP) family of bZIP proteins (C/EBP α , C/EBP β , and C/EBP γ) bind multiple members of the Rel family (p65, p50, and Rel). While C/EBP α and C/EBP γ function as corepressors (85), C/EBP β can either repress or synergize with NF- κ B. On the acute-phase response element of the rat angiotensinogen gene, NF- κ B and C/EBP β antagonize by competing for overlapping binding sites in the DNA (86), while on the promoters for IL-6 and IL-8, NF- κ B and C/EBP β activate transcription synergistically apparently by cooperative DNA binding (87, 88). Members of the signal transducers and activators of transcription (STAT) family of transcriptional regulators have also been shown to interact with NF- κ B. STAT6, normally a coactivator protein, has also been shown to function as a corepressor for NF- κ B, presumably by competing with NF- κ B for overlapping binding sites in the promoter region of the E-selectin gene (89). Conversely, STAT1 and NF- κ B

have been shown to synergistically activate transcription of proinflammatory genes in mouse fibroblasts, although the mechanism of this interaction is not well defined (90). The transcriptional coactivator protein CBP/p300 has been shown to interact with many transcription factors, and its interactions with p65 are a focus of much current research. Not only has CBP/p300 been shown to be a coactivator of p65-mediated transactivation (72, 91), but the specific interaction between p65 and CBP/p300 has been shown to be required for assembly of the "enhanceosome," an assembly of transcriptional cofactors that synergize and mediate transcription of interferon- β (75).

In addition to interaction with a variety of transcriptional cofactors, NF- κ B is also capable of interaction with other nuclear proteins that are themselves activatable transcription factors. An example of such an interaction is found between NF- κ B and AP-1 (Fos-Jun). When these two dimeric transcription factors interact via the Rel homology domain of p65 and the bZIP region of Fos and Jun, the transactivation function of each factor is potentiated (92). NF- κ B and the GR provide another example of NF- κ B/transcription factor interaction. For years, the mechanism by which glucocorticoids exerted their potent antiinflammatory effects was not understood. It is now clear that NF- κ B and the ligand-dependent GR can directly interact. In contrast to the potentiation of function with NF- κ B and AP1, the result of the GR/NF- κ B interaction is usually mutual transcriptional antagonism. (For angiotensinogen and probably several other hepatic acute-phase reactant genes, however, it appears that NF- κ B and GR positively interact at the acute phase response element to activate transcription (93–95).) NF- κ B/GR antagonism provides a fundamental mechanism for the regulation of many immune and inflammatory responses and therefore will be discussed in detail in a separate section of the text.

Other members of the steroid receptor family have also been found to interact with NF- κ B. Androgen receptor (AR) and NF- κ B have been shown to be mutual antagonists in cultured cell systems, and evidence for such antagonism in rat liver *in vivo* has also been demonstrated (4, 96). For progesterone receptor (PR) and estrogen receptor (ER), there is also evidence of negative interaction with NF- κ B, although the reciprocal antagonism of NF- κ B p65 by PR and ER appears to be a cell type-specific phenomenon (4, 97, 98).

F. Transgenic animals suggest a complex role for NF- κ B family members in immunity and development

As mentioned earlier, the generation of transgenic mice with deficiencies in specific NF- κ B subunits has underscored the importance of this family of transcription factors in development and the immune system. The phenotypic severity of the different knockout animals ranges from deficiencies in specific immune responses to embryonic lethality. The most dramatic Rel family knockout is p65/RelA, whose phenotype is embryonic lethal due to massive apoptosis in the liver. In addition, cultured embryonic fibroblasts from these animals demonstrate profound deficiencies in inducible NF- κ B activity, but unaffected basal NF- κ B activity. These findings point to p65 as being critical for activatable, but not constitutive, NF- κ B activity and suggests a significant role for p65

in both embryonic development and regulation of apoptosis (99).

The remainder of Rel knockout animals display normal embryonic development, but all show serious impairment of immune function. Immune deficiencies vary with each Rel protein knocked out, and in different cell types with a single type of Rel knocked out, supporting the idea of cell type specificities in the function and expression of the Rel family members. Knockouts of c-Rel (100) have profoundly impaired T cell function and proliferation as well as lymphoid hyperplasia. Knocking out Rel B results in multiorgan inflammation, T cell deficiencies, an inability to differentiate dendritic cells that cannot be compensated by expression of other Rel subunits, myeloid hyperplasia, and extramedullary hemopoiesis (28, 100). p50/p105 Knockouts exhibit B cells deficient in antibody production and proliferation (74). Interestingly, p105 knockouts (which express functional p50 but no inhibitory portion of the precursor molecule and consequently have enhanced p50 activity) suffer chronic organ inflammation, susceptibility to bacterial infection, and lymphoid hyperplasia. These different and opposing phenotypes in B cells, T cells, and macrophages suggest that the p50 homodimer functions as either a transactivator or transrepressor in a cell type-specific manner (101). Knockouts of p100 (which express functional p52 but no inhibitory portion of the precursor) die in the early postnatal period from gastric hyperplasia and exhibit overactive immune responses such as lymphocyte overproliferation and increased cytokine production (102). Animals that lack *bcl-3* expression can neither mount T helper responses nor antigen prime B or T cells (103).

The creation of double knockouts in Rel family members has revealed some unexpected phenotypes and suggests that Rel members may have redundant functions, again speaking to the importance of maintaining Rel function for proper immune response and development. For example, when p50 and p52 are both disrupted, mice are incapable of maturing osteoclasts and B cells. This leads to a phenotype of severe osteopetrosis and abnormal splenic/thymic architecture (104). Double p50/RelB knockouts die in the early postnatal period from massive organ inflammation that is far more severe than seen in a single-knockout animal (105).

III. Steroid Hormones/Receptors: Glucocorticoids and the Glucocorticoid Receptor (GR)

A. General background

Steroid hormones have long been recognized to be essential modulators of a wide array of cellular processes and intercellular communication, and the prominent role of steroids in the normal physiology and development of an animal have made them a focus of endocrine research since the middle of this century (reviewed in Ref. 106). While there is some evidence that "non classical" mechanisms of steroid hormone action may be important in special circumstances (106–108), the widely accepted, classical mechanism by which these lipophilic cellular signaling molecules are now understood to function is via a cognate cellular receptor molecule. These receptors bind steroid hormones and func-

tion as ligand-dependent modulators of gene expression. Until the mid-1980s, the majority of the knowledge concerning steroid hormone receptors was derived via standard biochemical techniques where the receptors were isolated and characterized using radiolabeled ligands (109). However, the field of steroid hormone receptor biology has rapidly accelerated since the successful cloning of the receptors (*e.g.*, Refs. 109–113) and the subsequent application of molecular biological techniques to gain an understanding of steroid hormone receptor function. The explosion of data in the steroid hormone receptor field over the past 15 yr has afforded us an understanding of the steroid receptors, their similarities and differences, and their role in transducing steroid hormone signals into cellular responses.

The steroid hormone receptors are a family of structurally similar, modular proteins that include GR forms α and β , PR forms A and B, mineralocorticoid receptors (MR), and ER forms α and β . Steroid hormone receptors belong to the nuclear receptor superfamily. All members of the nuclear receptor superfamily are believed to share a common ancestry, and all function via a similar mechanism of action, as ligand-dependent, DNA-binding transcription factors that interact with the basal transcriptional apparatus. Other classes of nuclear receptors include the thyroid, retinoid, and orphan receptors (114). Orphans are receptors with structural/sequence similarity to other nuclear receptors but for whom a ligand and/or function has not been identified.

Members of the steroid/thyroid/retinoid receptor superfamily share a variable amino-terminal transactivation domain, a central and well conserved DBD, and a moderately conserved carboxy-terminal domain responsible for binding ligand (110, 114). Within the steroid hormone receptor family, ER is the least conserved member. It has a highly homologous DBD but differs significantly from the other steroid receptors in primary structure outside this region. Because ER differs from the other steroid receptors and shares homology with thyroid hormone receptors, it is considered by some to be a separate subfamily within the steroid hormone receptor family. A schematic diagram comparing the primary structure of the steroid hormone receptors is found in Fig. 4.

B. Glucocorticoid mechanism of action: the GR

Since steroid hormone receptors function as transcriptional activators via a common mechanism, this general mechanism will be described using the α -form of the GR (GR α) as a specific example. As described above, GR α is a ubiquitously expressed (115) phosphoprotein of the steroid/thyroid/retinoid receptor superfamily. When GR α and other steroid receptors form high-affinity bonds with a cognate ligand, they interact with specific DNA sequences in the genome and function as transcription factors.

Glucocorticoids are lipophilic steroid hormones and, as such, are found in circulation associated with "carrier" proteins such as corticosteroid-binding globulin (in the case of endogenous steroids) and albumin (for both endogenous and synthetic steroids) (116). The most widely accepted mechanism for glucocorticoid entry into the cell is by free diffusion of these lipophilic molecules across the lipid bilayer of the

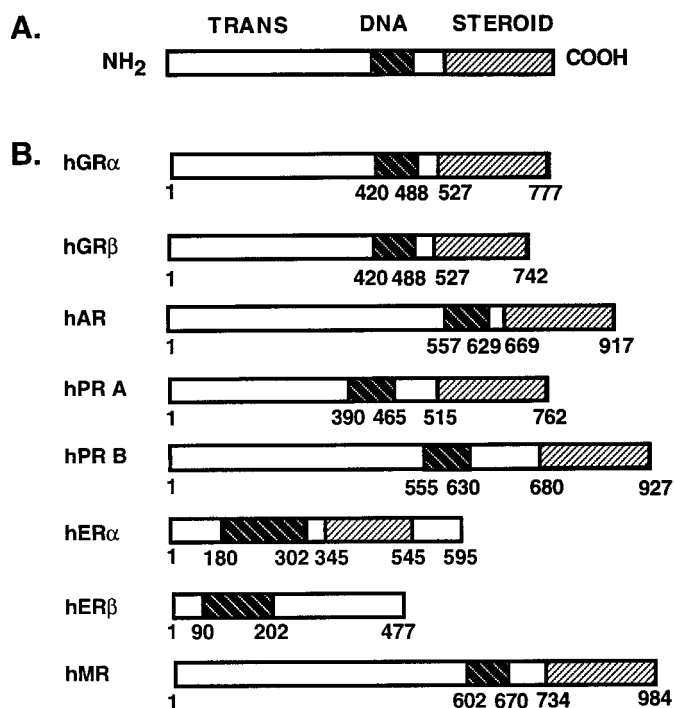


FIG. 4. Schematic diagram of the steroid hormone receptors: primary structure. A, The general structure of a steroid hormone receptor includes a variable transactivation domain in the amino terminus, a well conserved central DBD, and a moderately conserved steroid-binding domain in the carboxy terminus. B, Primary structure of each human steroid hormone receptor. GR α and GR β are the result of alternative splicing of the same mRNA transcript. PR A and B forms result from alternate sites of transcription initiation. ER α and ER β are believed to arise from separate genes. [Data are derived from Refs. 111–113 and 264–269.]

cell into the cytoplasm. However, there is some evidence (107, 108, 117) suggesting that glucocorticoid entry into cells is a regulated process involving specific membrane-associated receptors distinct from the classical intracellular GRs that are discussed in detail here. These membrane-associated receptors are believed to signal via G proteins and may also mediate the rapid, nongenomic effects of glucocorticoids that have been observed, particularly in neuronal tissues (108, 118).

In the absence of glucocorticoid hormone, classical GR α is retained in the cytoplasm in an inactive (*i.e.*, DNA binding-incompetent) state by its association with the regulatory heat shock proteins hsp 90 and hsp 56 (119, 120). The inactive conformation of GR exhibits high affinity for ligand. Once inside the cell, glucocorticoid binds the high-affinity cytoplasmic GR and induces a poorly understood process known as receptor activation. Activation of GR involves a change in receptor conformation, dissociation from regulatory heat shock proteins, and hyperphosphorylation. Activated receptor rapidly translocates to the cell nucleus and binds to specific DNA sequences as a homodimer (121–124). The DBD/dimerization domain of GR has been well characterized (125) and consists of two zinc ions coordinated with eight cysteine residues to form two peptide loops called zinc fingers. Each zinc finger is followed by an amphipathic α -helix. GR DBDs bind cooperatively to specifically spaced target half-sites in

the DNA, and this specific DNA association induces receptor dimerization. The subunits of GR then interact with adjacent major grooves of the DNA via their amphipathic α -helices. The specific palindromic sequences in the promoter regions of glucocorticoid-responsive genes to which activated GR dimer binds are known as GREs, or glucocorticoid responsive elements. The consensus GRE sequence is: 5'-GGTACA nnnTGTTCT-3' (126). Once bound to a GRE, homodimeric GR induces or increases transcription of the target gene, presumably by interacting with the basal transcription apparatus (127). In addition to its function as a ligand-dependent activator of transcription, GR also functions as a negative regulator of transcription in a specific subset of glucocorticoid-responsive genes. Many of these glucocorticoid-repressed genes contain a negative GRE (nGRE). nGREs are less well defined than positive GREs, and their gene-repressive function appears to be context dependent. The mechanism of action for GR on an nGRE likely involves displacement of a positive regulatory protein from the promoter (126, 128, 129).

Recently, research in our own and other laboratories has focused on a variant form of human GR, termed GR β , which arises from alternative splicing of the GR mRNA transcript. Originally cloned along with the GR α isoform in 1985 (130), GR β was largely ignored because it was not found to bind ligand or activate transcription of glucocorticoid-responsive reporter genes (130, 131). GR β is identical to GR α through the first 727 amino acids but diverges in the carboxy terminus, where it lacks the last 50 amino acids found in GR α but has an additional 15 nonhomologous amino acids past the point of divergence. As with GR α , GR β is widely expressed in adult and fetal human tissues but differs from GR α in that it is localized to the cell nucleus independent of the presence of ligand. The most recent results from transient transfection assays demonstrate that human (h) GR β functions as a dominant negative regulator of hGR α transactivation (Refs. 132 and 133; R. H. Oakley and J. A. Cidlowski, unpublished observations). Although the extent to which hGR β functions as a dominant negative *in vivo* is not yet clear (132–134), these studies suggest that tissue- or cell type-specific expression patterns of hGR β and hGR α may function to modulate the glucocorticoid responsiveness of tissues.

C. Glucocorticoid physiology

Glucocorticoids are synthesized in the zonae fasciculata/reticularis of the adrenal cortex and released into circulation in response to a wide range of stressful stimuli (*e.g.*, starvation, pain, surgery, trauma, emotional stress, extreme heat or cold, and cellular damage). Their release is orchestrated by the hypothalamic-pituitary-adrenal (HPA) axis, where hypothalamic CRH acts on the pituitary to cause release of ACTH, and ACTH then stimulates the adrenal gland to release glucocorticoid.

Glucocorticoids have a vast array of functions within the body, and an organism cannot survive without them (135). One of the first identified functions of glucocorticoids, and the one from which their name was derived, is as an important regulator of intermediary metabolism. Their main role in intermediary metabolism is to up-regulate the process of

gluconeogenesis in the liver, kidney, and skeletal muscle, which results in elevated levels of blood glucose. Glucocorticoids also modulate fat, protein, and glutamine metabolism, as well as bone turnover. The importance of glucocorticoids in regulation of metabolism is underscored by their role in the "metabolic syndrome," a collection of metabolic disorders including hyperlipidemia, hyperinsulinemia, insulin resistance, and hypertension, which is believed to be caused in part by hypersensitivity to cortisol (136, 137). Immune cell apoptosis is also known to be a glucocorticoid-regulated process (138), and there are glucocorticoid effects on the central nervous system that are as yet poorly understood. In addition to broad effects in the adult organism, glucocorticoids are known to have profound effects on fetal development and parturition and are especially important to fetal lung maturation.

Some of the most dramatic and clinically relevant effects of glucocorticoids are those on the immune system. Glucocorticoids are potent suppressors of immune response and inflammation. These characteristics have made synthetic glucocorticoids the drug of choice for therapeutic intervention in a broad range of autoimmune and inflammatory disorders. The benefits of glucocorticoids in treatment of rheumatoid arthritis were first recognized nearly 60 yr ago (139). Currently, a variety of synthetic glucocorticoids are employed in the treatment of systemic lupus erythematosus, inflammatory bowel disease, psoriasis, eczema, and asthma (3, 140–142). They are also used to suppress the host immune system and prevent rejection during organ transplantation. Interestingly, glucocorticoids enjoyed widespread use in the clinic, based primarily on empirical evidence of their efficacy, even before much information was available concerning their mechanisms of antiinflammatory and immunosuppressive action. Today, multiple mechanisms of antiinflammatory and immunosuppressive glucocorticoid action have been put forth in the literature, but the relative importance of these mechanisms is still unclear, and there is a good possibility that additional mechanisms are yet to be discovered.

The general mechanisms by which glucocorticoids exert their cellular effects have been elucidated over the past 15 yr, yet specifically understanding the immunosuppressive mechanism of glucocorticoid action has proven to be a conundrum. Although glucocorticoids are capable of reducing the number of lymphocytes both by redistribution of peripheral lymphocytes to the lymph nodes and by inducing lymphocyte apoptosis and growth suppression in the thymus (143–145), these functions are insufficient to explain the potent immunosuppressive action of glucocorticoids. Glucocorticoids are also known to suppress the expression of proinflammatory cytokines, which are key regulators of the immune response. As outlined above, glucocorticoids are now known to act via a cytosolic receptor that functions as a ligand-dependent transcription factor, modulating the expression of genes with GREs in their promoters. However, the majority of proinflammatory genes (such as cytokines) that are suppressed by glucocorticoids have no such responsive elements in their promoters, which might explain the role of glucocorticoids in their regulation. It was not until the role of NF- κ B in the immune system and interactions be-

tween NF- κ B and GR were identified that feasible mechanisms for the powerful glucocorticoid-mediated immune suppression could be proposed.

D. GR/NF- κ B interactions

GR activation results in altered expression of many genes that affect a variety of cellular processes. A listing of genes that are regulated by glucocorticoids can be found in Table 2. Brief inspection of Table 2 indicates that many genes involved in immune and inflammatory responses in the cell, particularly proinflammatory cytokines and cell adhesion molecules, are modulated by glucocorticoids.

The negative regulation of cytokines by glucocorticoids implicates them as important players in the process of GR-mediated immune suppression, yet the majority of these proinflammatory genes have no identifiable nGREs in them that can account for their susceptibility to GR-mediated repression. Interestingly, when one compares the NF- κ B-induced genes in Table 1 with the GR-repressed genes in Table 2, it is clear that NF- κ B and GR are physiological antagonists. A blockage of NF- κ B-mediated transcriptional activation by GR, rather than direct repression of proinflammatory genes by GR, could reconcile the GR-mediated repression of these genes with their lack of nGREs. For this reason, the interaction between NF- κ B and GR in immunity and inflammation has become an active and rapidly expanding area of research. Possible mechanisms of NF- κ B/GR antagonism are presented below.

1. Evidence for direct NF- κ B/GR interactions. It has been determined that GR and the p65 subunit of NF- κ B physically interact, and the consensus is that this (presumably direct) physical interaction involves the Rel homology domain of p65 and the DBD of GR (Refs. 1–4 and 146 and our unpublished observations). It has also been demonstrated that the physiological antagonism of GR and NF- κ B is based on a mutual transcriptional antagonism, rather than an alteration in the expression levels of these two transcription factors in the cell (4). Initial hypotheses concerning NF- κ B/GR antagonism focused on the possibility that the physical interaction between these two factors impaired the DNA-binding functions of each and therefore blocked the ability of each to activate transcription. However, subsequent studies on this question have provided contradictory results, and little conclusive evidence exists suggesting that the DNA-binding function of either transcription factor is impaired under conditions in which a mutual transcriptional antagonism occurs (2, 3, 147, 148). In fact, we have observed that NF- κ B p65 is capable of blocking GR transactivation of a reporter gene (4), but cannot interfere with homologous down-regulation of GR (a process that requires GR to bind intragenic GREs; J. C. Webster and J. A. Cidlowski, unpublished observations). This argues against a mechanism of repression that involves a simple blockage of DNA binding.

To further address the question of mutual transcriptional repression, other possible mechanisms that might drive the NF- κ B/GR antagonism were considered by several groups. One approach taken by our group was to functionally dissect both the GR and NF- κ B to determine which subunits/do-

main of these transcription factors are required for mutual transcriptional repression. A summary of these findings is presented in Fig. 5. First, it was determined that the p65 subunit, but not the p50 subunit, of the NF- κ B heterodimer could repress GR-mediated transactivation of a reporter gene. The ability of a series of GR deletion mutants (missing portions of the ligand-, DNA binding-, or transactivation domain) to negatively interact with p65 was then assessed. These experiments showed that multiple domains of GR are required to repress p65 transactivation. While the importance of the DBD of GR to the p65-GR interaction was already known (1, 3), the data demonstrated that deletion of either one of the two zinc fingers that are found in this domain, as well as deletion of the steroid-binding domain or large portions of the transactivation domain of GR, also abolishes the repressive effect of GR on NF- κ B. Interestingly, the reciprocal repression of GR by p65 was less selective, since all transcriptionally active mutants of GR were repressed by p65. These findings suggested that the reciprocal physiological antagonism between NF- κ B and GR might not be based on entirely reciprocal mechanisms of transcriptional antagonism. One mutant GR that was examined is both constitutively nuclear and transcriptionally active (since it has no ligand-binding domain and is ligand independent), yet it is still repressed by p65, suggesting that the mechanism of p65-GR antagonism does not involve cytoplasmic sequestration of the factors, but rather occurs within the nuclear compartment (4).

2. Evidence for cofactor involvement. The fact that mutual functional antagonism between GR and NF- κ B requires multiple domains of both proteins (1, 4, 146, 149), while physical interaction requires only a subset of those domains (the GR DNA-binding region and the p65 Rel region), argues against a simple mechanism of antagonism in which direct physical contact of GR and p65 is sufficient to prevent DNA binding and subsequent transactivation. More recent data suggest that the mechanism of mutual antagonism between NF- κ B and glucocorticoid-signaling pathways involves transcriptional cofactors that mediate the interactions of NF- κ B and the GR. The emerging evidence, which supports the idea of GR-cofactor interactions modulating GR signaling, is presented in detail in a separate section below.

One possible mechanism by which cofactors might mediate GR-p65 antagonism is as a target for competition. A limiting cellular pool of a common transcriptional cofactor, required by both GR and NF- κ B for full transactivation, might result in repression of one transcription factor's basal or induced activity when the other transcription factor was activated and had bound up all available cofactor. While no one cofactor has been identified that would occupy this role, one candidate currently being examined in both our own and other laboratories (146) is CBP/p300. The recent proliferation of yeast two-hybrid assays has demonstrated that many transcription factors (such as steroid hormone receptors and nuclear receptors), cofactors (such as SRC-1), and proteins of the basal transcriptional machinery (RNA polymerase II, TATA-binding protein, and TFII-B, for example) can interact with CBP, and a view of CBP as a central "adapter/integrator" protein, which brings a complex set of transcriptional

TABLE 2. Genes regulated by glucocorticoids/glucocorticoid receptor

Physiologic process system	Gene	Cell type/organ
INDUCED GENES		
Immune	I κ B α Haptoglobin TCR zeta p21 Cip1 (CDK inhibitor)	HeLa cells, lung epithelial cells, monocytes, T-cells Lymphocytes T cells Fibroblasts
Metabolic	PPAR γ PFK2 Tyrosine aminotransferase Glutamine synthetase Glycogen synthase Glucose 6-phosphatase PEPCK Tryptophan oxygenase Cytochrome p450 ob/leptin Glutaminase γ -Fibrinogen Cholesterol 7- α hydroxylase Argininosuccinate synthase S-adenosylmethionine synthetase Arginase	Adipose Liver Liver, lung Muscle, lung, brain Liver Liver Liver Brain Liver Adipose Liver, kidney Liver Liver Liver Hepatocytes Liver
Channels and transporters	Ileal bile acid transporter ENaC (sodium channels) α , β , γ Aquaporin 1	Ileum Fetal lung Mouse erythroleukemia
Other	GTP cyclohydrolase 1 Heme oxygenase bFGF Alkaline phosphatase Lipocortin β -Casein 5HT7 Adenosine A1 receptor Vasoactive intestinal peptide Endothelin RXR α -2 Microglobulin GHRH receptor α -1 Acid glycoprotein s100 β Androgen receptor Natriuretic peptide receptors Surfactant protein A Mouse mammary tumor virus (MMTV) C/EBP β IGF binding protein Calcitonin receptor	Adrenal medulla, PC12 cells Brain Hippocampal astrocytes Astrocytoma cells Mammary epithelium Cortical astrocytes Brain Pituitary Vascular smooth muscle Hepatocytes Liver Pituitary cells Liver Hippocampal astrocytes Osteoblasts Vascular smooth muscle Fetal lung Liver Multiple cell types Osteoclasts
REPRESSED GENES		
Immune	Interleukins 1,2,3,4,6,8,10,12 TNF α IFN γ E-selectin ICAM-1 ELAM-1 Cyclooxygenase 2 iNOS	Lung epithelium, peripheral lymphocytes, etc. Peripheral lymphocytes Peripheral lymphocytes Lung epithelium Lung epithelium Endothelial cells Multiple cell types Hepatocytes

Table was compiled from Refs. 77, 135, 148, 153, 190, 192, 203, 210, and 216–263.

TABLE 2. Genes regulated by glucocorticoids/glucocorticoid receptor (continued)

Physiologic process system	Gene	Cell type/organ
Endocrine	Glucocorticoid receptor	Multiple tissues and cell lines
	PRL	Pituitary
	POMC, (ACTH, β-endorphin, β-lipotropin)	Pituitary
	CRH	Hypothalamus
	PTHrP	Squamous epidermal cancer cell line
Developmental	Fibronectin	Placenta
	α-Fetoprotein	
	NGF	Hippocampal astrocytes
	Erythropoietin	Fetal kidney
Other	Tryptophan hydroxylase	Brain
	Vasopressin	Neurons
	Osteocalcin	Bone
	Transcription factor CBF1	Bone
	Collagenase	
	Metalloproteases	Multiple cell types
	β-Galactoside α2,6-sialyltransferase	Intestinal epithelium
	G1 cyclins	Multiple cell types
	Phospholipase A2	
	Cyclin-dependent kinases	
E4BP4	Fibroblast cell line	

Table was compiled from Refs. 77, 135, 148, 153, 190, 192, 203, 210, and 216–263.

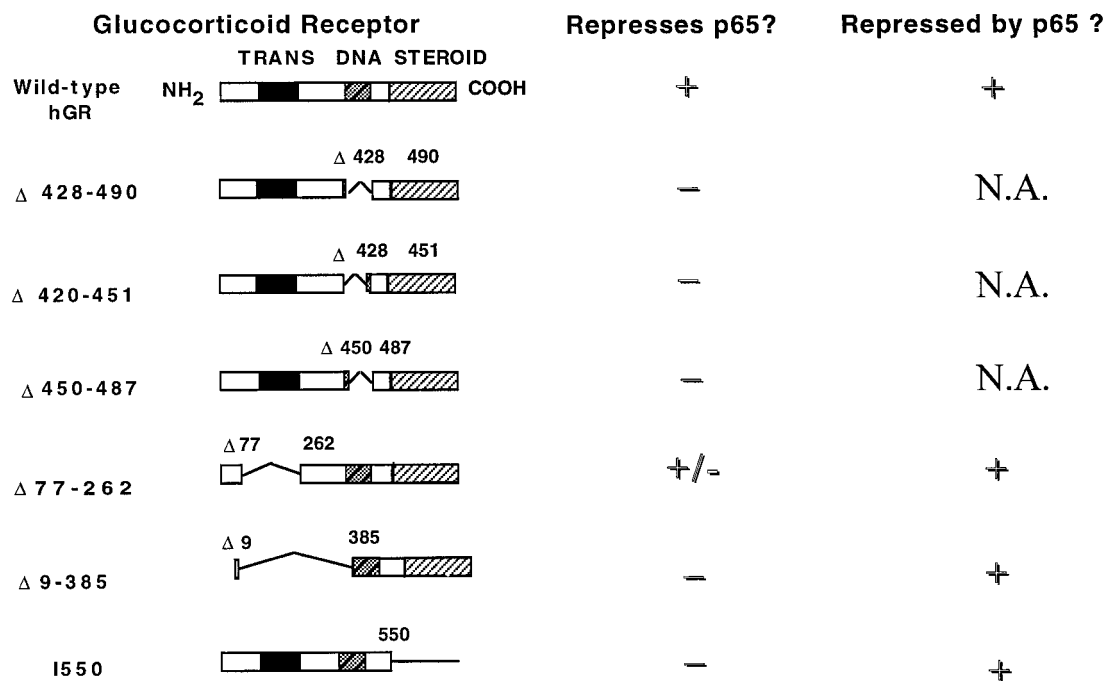


FIG. 5. Role of GR domains in p65/GR mutual transcriptional antagonism. Any transcriptionally active mutant of GR is repressed by p65. However, deletions in the GR DBD or the steroid-binding domain, as well as large deletions of the transactivation domain, all prevent GR-mediated repression of p65 transactivation.

proteins together on the DNA, is forming (78, 79, 150, 151). Both GR (see below) and NF-κB interact with CBP/p300, making CBP an attractive candidate for a role as mediator of NF-κB/GR antagonism, either by functioning as an adapter/cointegrator or as the limiting pool of a mutually required transcriptional cofactor. This idea of CBP functioning as a mediator of transcription factor antagonism was first pro-

posed by Kamei and co-workers (150), who provided evidence that nuclear receptor-mediated repression of AP-1 activation is a result of competition for limiting amounts of CBP.

While it seems logical to consider the role of cofactor (in particular, CBP) competition in NF-κB/GR antagonism, *in vitro* studies by Caldenhoven *et al.* (1) demonstrate that GR-

mediated repression of an NF- κ B reporter is overcome by increased expression of p65 and therefore argue against such a mechanism. Another potential problem with the hypothesis that CBP might be limiting is that activation of NF- κ B or GR should globally inhibit all receptors that require CBP for their activity. This simple model of transcription factor competition for CBP would apparently not allow for the specific nature of the NF- κ B/GR antagonism. However, the more complex proposal that CBP might function as a cointegrator and mediate the antagonism of NF- κ B and GR by bringing the two factors together in a specific inactive conformation is promising in that it allows for a specific repressive effect even when faced with both the relatively promiscuous interactions of CBP/p300 and the *in vitro* data that show that GR repression of NF- κ B can be titrated out by overexpression of p65.

3. Other mechanisms of NF- κ B/GR interaction. Another potential mechanism of NF- κ B/GR antagonism which does not involve direct physical interaction of GR and NF- κ B requires further examination. Recent work from our laboratory (J. C. Webster and J. A. Cidlowski, unpublished observations) indicates that NF- κ B increases the expression of hGR β , the endogenous dominant negative inhibitor of hGR α transactivation. Therefore, a cell in which NF- κ B is activated may have a higher ratio of GR β :GR α and, consequently, impaired GR transactivation. This would provide a dual mechanism of GR repression by NF- κ B: directly by mutual transcriptional antagonism and indirectly by increasing the relative amounts of a dominant negative regulator of GR. Such a mechanism would be reminiscent of a dual mechanism of NF- κ B repression by GR (directly by transcriptional antagonism and indirectly by inducing I κ B α), which has been identified and is discussed in Section IV of this manuscript.

4. Evidence for a functional antagonism of GR by NF- κ B. That two independent mechanisms of GR repression by NF- κ B likely exist within the same cell suggests that maintaining negative control on GR-signaling pathways is of physiological importance. Although most interest in NF- κ B/GR antagonism is focused on the role of GR in dampening NF- κ B actions, the antagonism of GR function by NF- κ B is probably equally important to the physiology of an organism. While not yet supported by direct evidence, it may be that glucocorticoid-responsive tissues with an activated inflammatory response (mediated by an activated NF- κ B) become somewhat resistant to glucocorticoid signaling because GR function is blocked.

The repression of GR by NF- κ B may also be relevant in patients with steroid-resistant asthma. The pathology of asthma is caused by chronic inflammation of the airway epithelium, and the treatment of choice for controlling this inflammation is administration of glucocorticoid (152, 153). However, a subpopulation of asthmatics do not respond to antiinflammatory treatment with glucocorticoids. Some of these steroid-resistant asthmatics have an abnormally low number of GRs, and others demonstrate reduced ligand binding affinity (presumably a reversible effect due to the actions of IL-1 and IL-4), which can explain the lack of glucocorticoid responsiveness (154, 155). Other steroid-resistant

asthmatics show no defects in their GRs or in steroid absorption or clearance (152). While there are data that suggest that abnormal AP-1 (activator protein 1)-GR interactions reduce GR DNA binding in these patients (152, 156), it will also be important to determine whether these individuals have any abnormality in NF- κ B regulation that leads to excessive NF- κ B activation. It is conceivable that a chronically high level of NF- κ B activity would lead not only to chronic inflammation, but also to glucocorticoid resistance by blocking of the GR-signaling pathway. Should abnormal NF- κ B regulation be found in these patients, targeting both NF- κ B- and GR-signaling pathways simultaneously could provide a promising approach to effective antiinflammatory therapy in this otherwise refractory population.

E. GR interacts with other transcription factors and transcriptional cofactors

As discussed, GR binds to specific regions of the chromatin and alters the basal level of transcription for responsive genes. However, as with NF- κ B, GR is now known to interact with a variety of nuclear regulatory proteins and other transcription factors that can modulate its function and alter its interaction with DNA and/or the basal transcription machinery. Interactions with different cofactors/transcription factors may contribute to cell type specificities in glucocorticoid responsiveness and GR-mediated gene expression.

1. Transcriptional cofactors and GR. Some proteins function as transcriptional coactivators for the GR, enhancing, or in some instances, enabling the transcriptional activity of ligand-activated GR. Examples of proteins recently identified as GR coactivators are CBP/p300 (cAMP-response element binding protein), GRIP1/TIF2 (GR-interacting protein-1 in mouse, transcriptional intermediary factor 2 in humans), HMG-1 proteins, 14-3-3 eta, STAT 5 (signal transducer and activator of transcription-5), GRIP 170, hRPF1, RAP 46, and SRC-1 (steroid receptor coactivator-1) (76, 78, 127, 157-165). Proteins that interact with steroid and other nuclear receptors and serve as transcriptional corepressors, including N-CoR (nuclear receptor corepressor) and SMRT (silencing mediator for retinoid and thyroid hormone receptors), have also been identified (78, 164, 166, 167). While it has not yet been conclusively determined whether any of these recently identified corepressors specifically interact with GR, it is likely that as more corepressors are identified and their mechanisms of action are clarified, corepressors that specifically affect GR-mediated transcription will be identified.

2. Transcription factors and GR. In addition to interacting with transcriptional cofactors, GR has been shown to interact with other proteins that are themselves independent transcription factors. This "cross-talk" has been shown to alter the transcriptional properties of both GR and the other transcription factors. For example, GR cross-talk is observed with members of the ubiquitous OTF (octamer transcription factor) family of constitutive transcriptional activators (168). Oct-1 and Oct-2 interactions with GR in some cell types and promoter contexts enhance the transcriptional activity of both factors via a cooperative binding mechanism, while in other contexts Oct-1/GR physical interactions result in functional

interference (169). Interaction with GR represses Oct-2A transactivation, possibly due to competition for transcriptional cofactors (170), and transcriptional repression of the bovine PRL (PRL3) gene by GR has been shown to be enhanced by interactions with Oct-1 and another homeodomain protein, Pbx (171). GR has also been shown to repress the activity of the transcription factor GATA-1 via direct physical interaction in mouse erythroleukemia cells (172) and to synergize with the developmentally regulated transcription factor hepatocyte nuclear factor-1 in liver and liver-derived cell lines (173).

3. *AP-1 and GR*. A particularly important physical interaction occurs between GR and the ubiquitous dimeric transcription factor activator protein-1 (AP-1). GR can interact with both Fos and Jun subunits of the AP-1 heterodimer (although it appears that Fos may be the preferential target for GR) and alter the interaction of both transcription factors with DNA, resulting in reciprocal repression of AP-1 and GR transactivation functions. GR also similarly interacts with Fos and Jun homodimers to alter transcriptional properties of both factors (174–179). AP-1 (Fos-Jun) is a proinflammatory transcription factor that is induced by a variety of cytokines and by phorbol ester (180). Like NF- κ B, AP-1 has been shown to activate transcription of genes involved in inflammatory diseases such as rheumatoid arthritis and asthma (180–182), and AP-1 and NF- κ B have been shown to act synergistically in the induction of some proinflammatory genes in lung epithelium (182). In addition, many inflammatory genes that are repressed by glucocorticoids but do not have nGREs in their promoters do carry sites for AP-1 as well as for NF- κ B (183). These data suggest that AP-1 repression by GR is another important mechanism of antiinflammatory and immunosuppressive action by glucocorticoids. It is important to bear in mind that, since NF- κ B and AP-1 can synergize in the proinflammatory pathway, GR repression of AP-1 may be an important indirect mechanism for suppressing NF- κ B-mediated immune responses.

4. *GR and other steroid hormone receptors*. It has also been demonstrated that GR can interact with other steroid receptors, resulting in altered transcriptional activity of genes responsive to each reporter. In some cases, the mechanism of interaction is known to involve heterodimerization. For example, GR and MR heterodimerize, and coexpression of GR and MR in a given cell may influence the cell type-specific pattern of gene activation (184). When AR and GR heterodimerize, mutual transcriptional inhibition results (185). GR and progesterone receptor A (PR-A) have also been found to interact. Although the mechanism of interaction is not yet clear, it has been demonstrated that PR-A can function as a dominant negative regulator of GR transactivation (186).

IV. NF- κ B and GR Antagonism: Physiological Significance?

NF- κ B is a key proinflammatory and proimmune transcription factor, and ligand-activated GR is a potent suppressor of immunity and inflammation. The negative crosstalk that has been demonstrated between these two

transcription factors with opposing functions has clear implications for the importance of these two transcriptional modulators in immune regulation. Given their profound effects on immunity and inflammation, it is conceivable that NF- κ B/GR mutual antagonism provides the primary control mechanism by which an organism maintains homeostasis in situations where host defense is activated. The signals that activate NF- κ B, such as bacterial or viral infection and oxidative stress, are perceived as stressful stimuli by the organism. These stimuli also activate the HPA axis, with the end result being the release of high levels of glucocorticoid into circulation. Therefore, both the pro- and antiinflammatory pathways are activated by these environmental stressors. The presumption is that GR-mediated immunosuppression serves to limit the cellular damage that would be caused by an excessive immune response.

While a strong case can be made for the importance of NF- κ B in glucocorticoid regulation of immunity and inflammation, it is important to maintain perspective and consider NF- κ B/GR effects within the context of all known GR-mediated effects on immune function. For example, AP-1 may also be a key mediator of these glucocorticoid effects. As discussed above, AP-1 regulates expression of proinflammatory genes both independently and synergistically with NF- κ B, and the well described antagonism of AP-1 by GR suggests that glucocorticoid effects on immune/inflammatory cascades are mediated through AP-1 dependent pathways in much the same manner that they are mediated by NF- κ B. AP-1/GR antagonism also suggests that proinflammatory genes whose transcription is synergistically activated by AP-1 and NF- κ B might be even more sensitive to glucocorticoid-mediated suppression than those that are activated by only one of these two transcription factors. There is also evidence that GR can directly regulate the expression of some proinflammatory cytokines and related genes via transcriptional and posttranscriptional mechanisms. For example, the IL-8 gene has been shown to be repressed in a fibrosarcoma cell line via a GRE in its 5'-flanking sequence (187), glucocorticoids dose-dependently destabilize IL-6 mRNA via a receptor-mediated pathway (188), and a novel GRE has been identified in the murine IL-2 receptor α gene (189). Therefore, it is likely that multiple mechanisms contribute to the potent immunosuppressive and antiinflammatory effects of glucocorticoids *in vivo*. However, the interaction between NF- κ B and GR is multifaceted, involving mutual transcriptional antagonism, feedback mechanisms involving cytokine cascades, and regulation of I κ B, which is discussed below. Multiple mechanisms of antagonism afford multiple sites of regulatory control and flexibility of the interaction. Such flexibility and capacity for precision regulation make NF- κ B an attractive candidate for the primary mediator of glucocorticoid effects in the immune system.

As discussed, an additional level of control of NF- κ B by GR involves regulation of I κ B α expression. I κ B α , the inhibitory subunit of NF- κ B, has been shown to be up-regulated by active GR (190–192). This positive regulation of I κ B α by GR presumably dampens proinflammatory effects of NF- κ B and therefore is another mechanism by which glucocorticoids exert their antiinflammatory effects, keeping immune responses in check. The extent to which this second mechanism

of NF- κ B regulation by GR influences immune function is an area that requires further study. Since NF- κ B is a family of transcription factors with differing responsiveness to activating pathways and I κ B α repression, as well as different cellular distributions, induction of I κ B α by GR cannot account for all of the antiinflammatory effects of glucocorticoids. Recent studies by Heck *et al.* (193) support this idea. They demonstrate that a dimerization-deficient GR mutant represses NF- κ B yet cannot induce I κ B α expression. In addition, they show that some glucocorticoid analogs can activate I κ B α but do not repress NF- κ B. Thus, I κ B α induction by GR is a separable process from GR-mediated repression of NF- κ B transactivation. The fact that separate and concurrent pathways for repression of NF- κ B by GR exist suggests that it is necessary for an organism to keep immune and inflammatory responses under tight control, and that glucocorticoids are an important physiological means of maintaining this control.

The idea that NF- κ B is an important physiological repressor of GR-mediated cellular processes is less well developed, but this may prove to be an equally important result of GR/NF- κ B cross-talk. The question of whether prior activation of NF- κ B in a normally steroid-responsive cell confers a degree of glucocorticoid resistance requires further study. It may be that the efficacy of pharmacological doses of glucocorticoid for control of inflammation is modulated by the level of NF- κ B expression in the target cell. Or, a state of inflammation (and elevated NF- κ B activation) might impinge on other functions of the glucocorticoid-mediated signaling pathway that occur at physiological levels of the hormone, thus influencing normal metabolic or developmental processes, for example.

The role of NF- κ B/GR interactions in nonimmune processes and cell types is not yet clear. We know that both NF- κ B and GR enjoy broad ranges of expression within the various tissues of an organism, and that these two factors are often expressed in the same cell types. Whether they are mutual antagonists in nonimmune cells is largely a function of whether both pathways would be activated simultaneously under any circumstances. No definitive evidence exists that, outside the immune system, a balance between GR- and NF- κ B-mediated signaling is physiologically relevant in an intact animal. However, since such a balance is mimicked in cultured COS-1 cells (4), the possibility that some states of steroid resistance are mediated by NF- κ B should be considered. One might also speculate that NF- κ B/GR antagonism might be important in regulating apoptosis or programmed cell death. GR is known to be an inducer of thymocyte apoptosis (194), although its role in apoptosis in other cell types has not been established. NF- κ B p65 knockout mice are embryonic lethal due to massive liver apoptosis (99), suggesting a possible role for NF- κ B as a general suppressor of apoptosis. Whether these roles as apoptotic inducer and suppressor, respectively, are simultaneously filled in any one cell type is as yet an open question.

V. Interactions Between NF- κ B and Other Steroid Hormone Receptors

Within the immune system, a homeostatic role for this negative cross-talk between NF- κ B and GR has been iden-

tified. That interactions between NF- κ B and GR might also be relevant in other physiological situations is a logical assumption given their widespread distribution and broad range of regulated genes. Ongoing research concerning NF- κ B and GR function outside the immune system should clarify this issue.

The great structural similarity of GR to the other members of the steroid hormone receptor family, as well as the fact that they share a common mechanism of action as ligand-dependent transcription factors, begs the question of whether other steroid hormone receptors (such as estrogen, progesterone, or androgen) might also interact with NF- κ B via mechanisms like those proposed for GR. While our understanding of this question is still sparse, evidence that such interactions do occur is beginning to emerge and is summarized below.

A. Androgen receptor (AR)

AR is closely related to GR in terms of both structure and sequence specificity (they share 78% sequence identity in their DBDs). GR and AR have been shown to interact with and repress AP-1 via similar mechanisms (195). Therefore, it would not be surprising that AR might also interact with NF- κ B in a manner similar to that observed for GR. Transient cotransfection experiments performed in our laboratory indicate that AR and NF- κ B (p65) are mutual transcriptional antagonists (4). While the *in vivo* significance of this antagonism has yet to be established, it is possible that inflammatory agents that activate NF- κ B *in vivo* may interfere with normal androgen signaling, thus producing androgen insensitivity in tissues where AR is normally expressed and exposed to ligand. Since we have shown AR/NF- κ B antagonism to be mutual, one might also speculate that androgen-responsive tissues might be relatively insensitive to NF- κ B-induced inflammation.

Interestingly, Supakar *et al.* (96) have observed physical interaction of NF- κ B p50 and p65 subunits with the promoter region of the AR gene *in vitro* (by electrophoretic mobility shift assay). This interaction of the NF- κ B and AR signaling pathways is different than any identified for GR, and it will be interesting to determine whether a similar mechanism for GR/NF- κ B antagonism can be identified. They suggest that age-dependent androgen insensitivity of the liver may be related to increased levels of p50 homodimer expressed in aging liver, which bind to the AR gene and subsequently block the androgen-signaling pathway. The extent to which the *in vitro* interactions of AR and NF- κ B reflect physiological interactions that occur within an organism remains to be seen.

Another interesting consideration for future research into NF- κ B/AR cross-talk is the ability of AR and GR to heterodimerize (185). Since both these receptors antagonize NF- κ B as homodimers, it is of interest to consider whether they maintain this function as a heterodimer, and whether cofactor or cell type specificities change under these circumstances.

B. Estrogen receptor (ER)

Several groups (4, 84, 92, 196) have shown that NF- κ B p65 and ER interact. Interestingly, while all groups show that p65

represses ER α -mediated transactivation in transient cotransfection assays, whether or not this transcriptional antagonism is reciprocal is less clear. Our studies using COS-1 cells indicate that ER is incapable of reciprocally repressing NF- κ B signaling. Stein and Yang (98) show a mutual antagonism in some cell lines and one-way antagonism in others. A more recent report assessing the regulation of NF- κ B signaling in cell lines confirms that ER antagonizes NF- κ B in a cell type-specific manner consistent with a cofactor-modulated process but could not demonstrate a role for known ER cofactors in this specificity (197). This suggests that for ER, there may be cell type-specific cofactors that are important modulators of the ER/NF- κ B interaction but have not yet been identified.

While the exact mechanisms of ER/NF- κ B antagonism are not yet clear, the physiological importance of these interactions is suggested by several recent reports. For example, estrogens are known to be key regulators of bone metabolism, and their withdrawal is related to the bone mineral loss of osteoporosis. It has been shown by several groups that this bone-protective effect of estrogen is due, at least in part, to estrogen-mediated repression of IL-6, a cytokine that is transcriptionally up-regulated by NF- κ B and regulates bone metabolism (196, 198, 199). While each of these groups attributes this IL-6 inhibition to ER interference with NF- κ B binding to the IL-6 promoter, the mechanism by which ER blocks NF- κ B binding is not agreed upon. Galien and Garcia (196) show evidence for ER/NF- κ B complex formation that prevents DNA binding, while Kurebayashi *et al.* (198) assert that ER cannot prevent NF- κ B DNA binding. Sun *et al.* (199) show that estrogen blocks I κ B α degradation, suggesting that the inhibitory effects of estrogen on NF- κ B function are due to the sustained presence of the inhibitory subunit of NF- κ B.

ER/NF- κ B interactions have also been implicated in the progression of breast cancer. Nakshatri and co-workers (58) show that progression of a mammary carcinoma cell line from ER-positive nonmalignant to ER-negative metastatic correlates with constitutive activation of NF- κ B and altered expression of both NF- κ B and I κ B. They hypothesize that, since ER represses both constitutive and inducible NF- κ B activity, overexpression of NF- κ B-inducible genes in ER-negative cells contributes to malignancy and chemotherapeutic resistance. Multiple mechanisms for the interaction of ER and NF- κ B in this breast cancer model have been proposed. In addition to the previously described mechanisms of 1) repression of NF- κ B DNA binding by physical association with ER, and 2) the regulation of I κ B expression by estrogen, these authors propose that ER might interfere with NF- κ B signaling by increasing expression of PR. As described in the subsequent section, PR has also been shown to negatively interact with NF- κ B.

C. Progesterone receptor (PR)

As with ER, both our group and others have demonstrated a repressive effect of p65 on PR (B-form) transactivation, and the physical interaction of NF- κ B and PR has also been demonstrated *in vitro* (4, 97). Again, it is less clear whether PR reciprocally represses NF- κ B transactivation, and as with ER/NF- κ B mutual antagonism, this may be dependent on the cell type in which the interaction occurs. No clear phys-

iological role for NF- κ B/PR antagonism has been elucidated. However, it has been proposed that, since most progesterone target tissues (such as breast and endometrium) express receptors for cytokines that regulate NF- κ B, there might be a physiological relevance to p65/PR antagonism in normal cellular processes in these cells. Clearly, much more data concerning this question must be available before a firm role for PR/p65 antagonism can be postulated.

While it is not clear that the mechanisms of mutual antagonism are the same for GR and NF- κ B as they are for the other steroid hormone receptors and NF- κ B, it is logical that the physiological result of the antagonism would be similar: a cell type-specific homeostasis brought about by opposing transcriptional effects on a gene or group of genes. The cell type specificity of response would depend on the cofactors present within a cell, as well as the pattern of expression for NF- κ B and steroid hormone receptors in that cell type. Even if all necessary transcriptional factors are present within the cell, a physiologically relevant antagonism would depend on the temporally coordinated activation of both transcription factors by ligand or extracellular signal. Whether such conditions exist for these steroid and other nuclear receptors with NF- κ B in a single cell *in vivo* remains to be shown. If they do, this may present an important mechanism by which cells may become steroid resistant. At present, more questions than answers are available concerning NF- κ B interaction with the steroid hormone receptor family. For NF- κ B and GR in the immune system, however, evidence is strong that the interactions of their signaling pathways provide a fundamental homeostatic control mechanism.

VI. Summary/Conclusions

Immunity and inflammation are physiological processes of profound importance to an organism: without these processes, a host would quickly succumb to invading pathogens or damaging stimuli, yet excessive or inappropriate activation of these responses causes tissue/cellular damage and even death. Therefore, maintaining immune homeostasis is critical for the survival of an organism.

Both pro- and antiinflammatory mechanisms must be present and functional for a cell (and by extension, an organism) to survive in the face of environmental stimuli that elicit an immune response. These pathways, when activated simultaneously, provide homeostasis by "pulling" the cell in opposite directions. Two candidate signaling pathways for this physiological balancing act are the NF- κ B and GR-mediated signaling pathways. These pathways, as discussed in this review and depicted in Fig. 6, have important and opposing roles in immune function. NF- κ B is a transcription factor which induces the expression of many genes that participate in immune and inflammatory responses, while GR is a transcription factor that serves as a potent antiinflammatory agent and immune suppressor. Their interactions within the cell, while not yet completely understood, appear to be an important, possibly even the primary, mechanism of immune homeostasis. For this reason, research on GR-NF- κ B interaction continues to expand as many groups strive to fill in the "black boxes" where the GR and NF- κ B-signaling pathways cross.

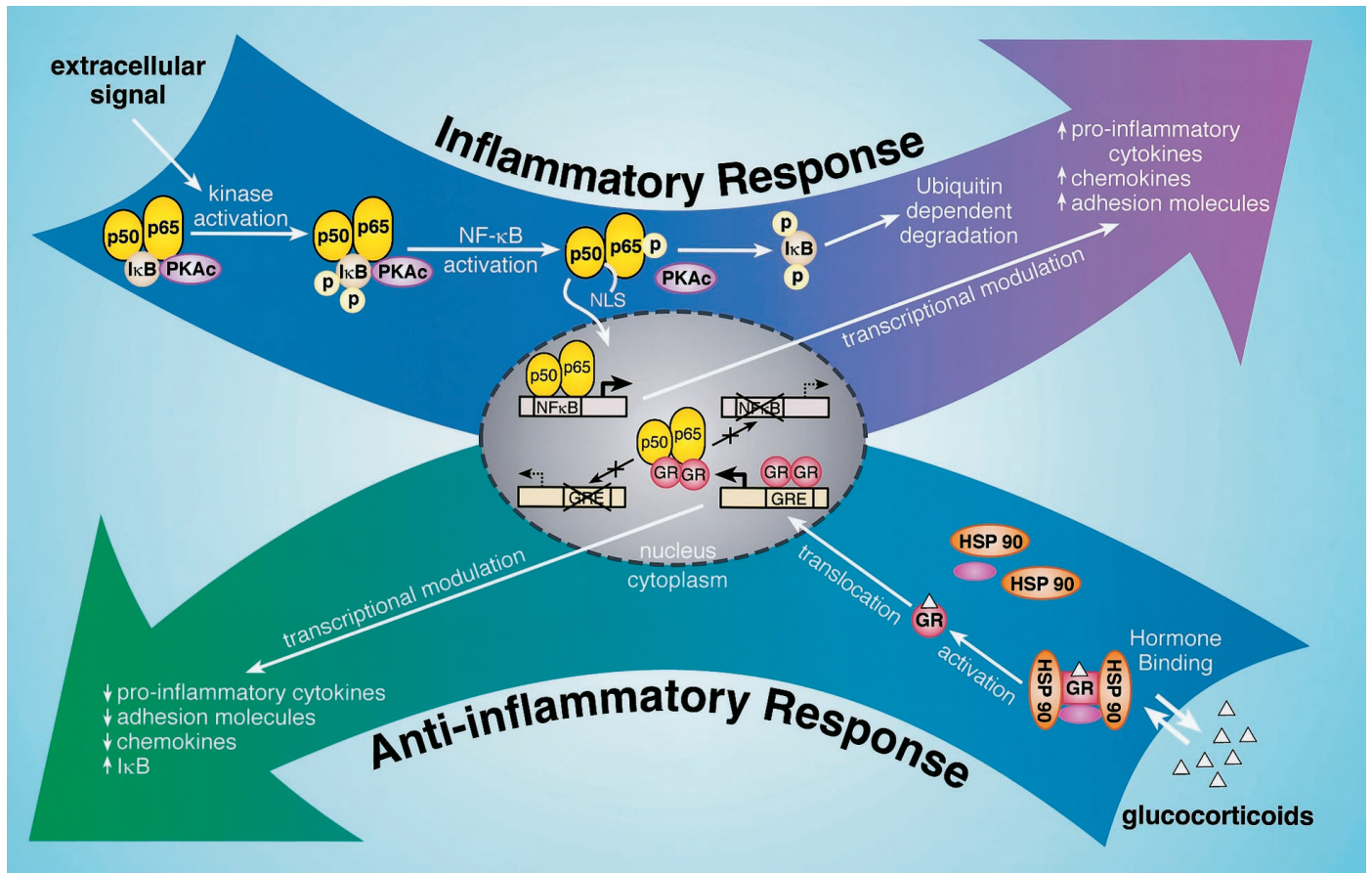


FIG. 6. NF-κB and GR as physiological antagonists in the process of inflammation. The NF-κB-signaling pathway is an important proinflammatory mechanism, whereas glucocorticoids acting via the GR-signaling pathway are potent suppressors of inflammation. The points of intersection between these two physiological mediators of inflammation is a popular topic of current research.

The interaction of inducible/activatable transcription factors, such as NF-κB or the steroid hormone receptors, with the basal transcriptional machinery is an important mechanism for tightly regulating gene-mediated physiological processes such as immunity and inflammation. However, the mechanism by which GR elicits its antiinflammatory effects has, until recently, proven to be a conundrum, since the majority of proimmune/proinflammatory genes repressed by glucocorticoids have no identifiable GREs in their promoters that would allow GR to interact with the basal transcription machinery. The relatively recent discovery that GR and NF-κB are mutual transcriptional antagonists has given researchers a handle on potential mechanisms of immune regulation by glucocorticoids.

The functional interaction of NF-κB and GR has thus far been demonstrated only in *in vitro* and cell culture systems, so it is important to consider whether these regulatory factors interact in precisely the same way within a more "physiological" context. Clearly, the field of NF-κB/GR research must move toward understanding how these two factors interact in an intact animal. However, understanding how such complex and broadly used signaling pathways interact within an intact organism is a daunting task. As discussed in this review, both NF-κB and GR have cell/tissue-specific effects, and both cross-talk with other steroid hormone re-

ceptors, transcription factors, and a variety of other cellular signaling cascades that are functioning simultaneously within an animal. The design of tissue-specific transgenic or inducible NF-κB and GR knockout animals would provide a powerful tool for understanding functional interactions of NF-κB and GR in immunity and inflammation (as well as other processes mediated by these two factors) in a more physiological context. Given our present scientific tools, however, the most valuable means of understanding NF-κB/GR cross-talk is probably still to examine the interaction in a relatively artificial context where a single pathway or response can be evaluated.

Despite the limitations of currently available model systems, our understanding of the elegant interplay between NF-κB and GRs in terms of immune regulation is rapidly improving. Gaining insight into mechanisms of NF-κB/GR antagonism not only opens up the possibility of novel, transcription-based therapy in immune pathology, but also raises the exciting possibility that NF-κB may also interact with GR or another steroid hormone receptor in other cell types to regulate other important physiological and developmental processes. As the body of research concerning NF-κB/GR expands, we may find that GR/NF-κB antagonism in the immune system is simply the prototype of a fundamental NF-κB/steroid receptor factor cross-talk mechanism that af-

fords tight homeostatic control of critical cellular processes in a variety of cell types.

Preliminary studies indicate that NF- κ B also interacts with androgen, estrogen, and PRs. These findings suggest that we may find sex-specific differences in immunity or other NF- κ B-mediated responses in an organism due to cross-talk with gonadal steroid-mediated signaling pathways. It also suggests the existence of a general nuclear receptor/NF- κ B cross-talk mechanism that may exert widespread influence on cell type-specific gene transcription. In light of this possibility, continued research into the mechanism of GR/NF- κ B antagonism is truly exciting.

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Addendum/Erratum

In the June 1999 *Endocrine Reviews* article by John F. Couse and Kenneth S. Korach, "Estrogen receptor null mice: what have we learned and where will they lead us?", Section II lists three references to the 1996 discovery of ER β . One of the references, Ref. 50, was actually published in 1997. To clarify this point, the discovery of ER β in the rat was first described by the Gustafsson laboratory in March 1996 and reported in June 1996 (Ref. 49). The human ER β was subsequently reported later that year (Ref. 51). The report of the mouse ER β was not reported until early 1997 (Ref. 50).