REVIEW

Negative regulation of nuclear factor-κB activation and function by glucocorticoids

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Abstract

Glucocorticoids (GCs) exert their anti-inflammatory and antiproliferative effects principally by inhibiting the expression of cytokines and adhesion molecules. Mechanistically, GCs diffuse through the cell membrane, and bind to their inactive cytosolic receptors (GRs), which then undergo conformational modifications that allow for their nuclear translocation. In the nucleus, activated GRs modulate transcriptional events by directly associating with DNA elements, compatible with the GCs response elements (GRE) motif, and located in variable copy numbers and at variable distances from the TATA box, in the promoter region of GC-responsive genes. In addition, activated GRs also acted by antagonizing the activity of transcription factors, in particular nuclear factor-κB (NF-κB), by direct and indirect mechanisms. GCs induced gene transcription and protein synthesis of the NF-κB inhibitor, IκB. Activated GR also antagonized NF-κB activity through protein–protein interaction involving direct complexing with, and inhibition of, NF-κB binding to DNA (Simple Model), or association with NF-κB bound to the κB DNA site (Composite Model). In addition, and according to the Transmodulation Model, GRE-bound GR may interact with and inhibit the activity of κB-bound NF-κB via a mechanism involving cross-talk between the two transcription factors. Lastly, GR may compete with NF-κB for nuclear coactivators, including CREB binding protein and p300, thereby reducing and inhibiting transcriptional activation by NF-κB. It should be noted that, in exerting its effect, activated GR did not affect the correct assembly of the pre-initiation (DAB) complex, but acted rather more proximally in inhibiting the correct assembly of transcription factors in the promoter region, and thus transcriptional initiation.

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Introduction

Glucocorticoids (GCs) are clinically used in treating disorders of heightened immunity, including transplantation rejection and autoimmunity (Almawi *et al.* 1998*a*). Despite a vast literature on their effects on T cell activation, the mechanism of action of the GCs remains incompletely understood. It is now recognized that their mode of action is multifaceted, since evidence of blockade of T cell immunity by the GCs at several stages in the T cell activation cascade is well documented (Almawi *et al.* 1996*a*, 1998*a*). The major routes by which GCs mediate their effects are many, the most significant of which are inhibition of cytokine production (Kwon *et al.* 1994, Mori *et al.* 1997, Almawi *et al.* 1998*a*) and, for some cytokines, signaling through their high-affinity receptor complex (Paliogianni *et al.* 1993, Monfar & Blenis 1996, Sakai *et al.* 1999, Almawi & Tamim 2001). Paradoxically, GCs upregulated the expression of some (proinflammatory) high-affinity cytokine receptors on target cells in the face of lost cytokine stimulation (Almawi *et al.* 1998*b*, Lukiw *et al.* 1999).

Originally, it was postulated that this inhibition involved binding of the hormone-activated GC

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receptor (GR) complex with specific DNA sites, the glucocorticoid response elements (GRE), which are located in variable copy numbers and at variable distances from the TATA box in the promoter of GCs-regulated genes, including cytokine genes (Almawi *et al.* 1990). Recent evidence suggested an additional mechanism, namely antagonism of transcription factors, in particular nuclear factor-κB (NF-κB), which resulted in attenuation or arrest of transcriptional activities (Auphan *et al.* 1995, De Bosscher *et al.* 1997). However, it is very likely that GCs may utilize more than one mechanism in exerting their anti-proliferative effect (Almawi *et al.* 1998*a*). This paper focuses on the effect of GCs on downregulating NF-κB activity; for discussion of the effect of GCs on antagonism of other transcription factors the reader is advised to consult excellent reviews published elsewhere (Gottlicher *et al.* 1998, De Bosscher *et al.* 2000*a*).

An overview of NF-κB and its role in T cell activation

Activation of T cells by ligating the T cell receptor (TcR) and the CD3 complex to foreign peptide–MHC class II complex bound on antigen-presenting cells (signal 1) (Wange & Samelson 1996, Madden 1995) is a highly ordered process, which involves activation of downstream intracellular target molecules and induction of cellular activation. Optimal T cell activation requires, in addition, the provision of costimulatory signals imparted by CD4/CD8 coreceptors, CD28 (Lucas & Germain 2000), and cytokine receptors (Curtsinger *et al.* 1999) among others (signal 2), which synergize with primary calcium-dependent TcR-CD3 ligation (signal 1), resulting in induction of the interleukin (IL)-2 autocrine pathway (Germaine 1994), and the temporal expression of cytokine genes and high affinity cytokine receptors (Curtsinger *et al.* 1999, Slifka & Whitton 2000). Signaling through the antigen-specific TcR in conjunction with non-cognate costimulatory signals results in the elevation of intracellular calcium and the induction of calmodulin-regulated enzymes, including the serine-threonine phosphatase, calcineurin (Clipstone & Crabtree 1992). In addition, protein kinase C (PKC) becomes activated, and translocates from cytosolic to membrane-bound compartments where it expresses its enzymatically active conformation. This induces the activation and nuclear translocation of the nuclear factors (nuclear factor of activated T cells (NF-AT)) (induced by calcineurin), and NF-κB where they bind the IL-2 enhancer and stimulate IL-2 transcription (Angel & Karin 1991, Blank *et al.* 1992).

NF-κB, a member of the mammalian rel gene family which comprises p105/p50, p100/p52, p65 (RelA), RelB, and c-Rel (Baeuerle & Baltimore 1996), is a heterodimer of p65 (RelA) and p50, and in the inactivated state is sequestered in the cytoplasm through the ankyrin repeats of its specific inhibitor, IκB. IκB, a member of a family of 7 inhibitory molecules that comprises IκBα, IκBβ, IκBε, and IκBγ and others (Ghosh *et al.* 1998), masks the nuclear localization signal of NF-κB, resulting in its retention in the cytoplasm. Activation by extracellular signals induces phosphorylation and ubiquitinylation of IκB by specific IκB kinases (IKKα and IKKβ), leading to its rapid proteolytic degradation, and thus the release of NF-κB (Cohen *et al.* 1998, Ghosh *et al.* 1998). NF-κB then undergoes nuclear translocation and binds its decameric DNA response element as a homo- or heterodimer comprising p50 and p65 (RelA) subunits, thus stimulating the transcription of NF-κB-regulated genes (Baeuerle & Baltimore 1996, Ghosh *et al.* 1998), including cytokine and IκB genes (Beg & Baldwin 1993, Scheinman *et al.* 1995). Persistent NF-κB activation, in turn, leads to increased IκB synthesis, and sequesters cytosolic NF-κB thereby attenuating its activity (Beg & Baldwin 1993, Baeuerle & Baltimore 1996).

Glucocorticoid antiproliferative effects

The anti-proliferative effect of GCs results from inhibition of cytokine expression at the transcriptional and post-transcriptional levels (Almawi *et al.* 2002). GCs inhibited the expression of proinflammatory and immunoregulatory cytokines, including IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-8, IL-11, IL-12, IL-15, IL-16, interferon-γ, tumor necrosis factor-α (TNF-α), and the colony-stimulating factors (CSF) macrophage (M)-CSF, granulocyte (G)-CSF, CSF-1, and granulocyte-macrophage (GM)-CSF (reviewed in Almawi *et al.* 1996*a*, 1998*a*). Inhibition by GCs of cytokine expression was specific for the GCs, as evidenced by the failure of non-GC steroids to inhibit cytokine expression

(Boumpas *et al.* 1993) or T cell proliferation (Almawi *et al.* 1996*b*), and by the capacity of the GCs receptor (GR) antagonist, RU-486, to abrogate the effects of GCs (Paliogianni & Boumpas 1995, Almawi & Tamim 2001). Inhibition of cytokine expression by GCs occurred at the transcriptional and post-transcriptional levels (Almawi *et al.* 2002), and was antagonized by exogenously reconstituted cytokines, which abrogated GCs-mediated effects, including induction of apoptosis (Mor & Cohen 1996), and suppression of cytokine production (Haynesworth *et al.* 1996) and T cell proliferation (Almawi *et al.* 1991).

Molecular mechanism of glucocorticoid action

Owing to their lipophilic nature and low molecular weight, GCs diffuse through the membrane lipid bilayer, where they bind their intracellular receptor (GR), a hormone-activated, dual zinc finger transcription factor. Depending on the target gene, ligand-activated GRs may stimulate (*transactivation*), or alternatively inhibit (*transrepression*) gene transcription. The former is exemplified by the capacity of GCs to upregulate the expression of the specific NF-κB inhibitor, IκB (Auphan *et al.* 1995, Scheinman *et al.* 1995). The latter is evidenced by the well-documented capability of GCs in inhibiting the expression of IL-2 and other cytokine genes (Almawi *et al.* 1991, Mori *et al.* 1997). When not bound to its ligand, the GR is sequestered in the cytoplasm as an inactive complex with two molecules of heat shock protein (HSP-90), and other cytosolic proteins (Oakley *et al.* 1999).

Upon binding GCs, GR undergoes conformational changes, which allow it to dissociate from HSP-90 molecules. The hormone-bound GR then translocates to the nucleus, where it transiently associates with another heat-shock protein, HSP-56, and later dissociates from HSP-56 and binds as a dimer to conserved palindromic DNA sequences, named the GCs response elements (GRE). These comprise two boxes spaced by three variable nucleotides (GGTACAnnnTGTTCT), each box interacting with one of the two GR zinc fingers (Berg 1989, Miesfield 1990). GREs are located in variable copy numbers, and are found at variable distances from the TATA box in the promoter region of GCs-responsive genes, including cytokine genes (Almawi *et al.* 1990). Furthermore, depending on the target gene, binding of the GR to GRE sites may enhance or repress transcriptional activity (Northrop *et al.* 1992, Paliogianni & Boumpas 1995), and two classes of GREs mediate the effects of GCs: stimulatory GRE (sGRE) and negative GRE (nGRE), the former responsible for GCs stimulation, while the latter mediates GCs inhibition of gene expression. This confers a dual transcriptional modulatory capacity on the GR.

The GR is a member of the steroid superfamily which comprises steroid, thyroid hormone, vitamin D, and retinoic acid receptors (Evans 1988), and consists of 3 domains: a hormone (steroid)-binding domain, a highly conserved DNA-binding domain, and the least conserved N-terminal region (Evans 1988). Binding of hormone-activated GR to GRE elements results in blockade of transcriptional activity in a *cis*-acting (De Bosscher *et al.* 2000*a*) or a *trans*-acting fashion which involves induction of the expression of specific GCs-associated inhibitor(s). According to the former, GR-GRE binding involves masking of the DNA binding sites of basal (Ray & Sehgal 1992) and induced (Akerblom *et al.* 1988, Mordacq & Linzer 1989) transcription factors. The latter mechanism postulates the expression of a specific GC-induced inhibitor of T cell activation (Samuelsson *et al.* 1999), including the NF-κB antagonist IκB (Auphan *et al.* 1995). While evidence supporting both scenarios is documented, conclusions drawn must be viewed in the context of the cell type and gene studied.

Antagonism of NF-κB activation and function by glucocorticoids

In addition to the GR-GRE interaction model, GCs reportedly repressed gene expression by antagonizing transcription factor activity and/or function. This was evidenced by the capacity of hormone-activated GR to repress the nuclear translocation and/or function of the transcription factors AP-1 (a dimer of c-Fos and c-Jun) (Vacca *et al.* 1992, Mori *et al.* 1997), NF-κB (De Bosscher *et al.* 1997, 2000*b*, Heck *et al.* 1997), and NF-AT (Northrop *et al.* 1992, Paliogianni *et al.* 1993, Chen *et al.* 2000). Reduction in transcription factor availability and/or function, in turn, resulted in downstream reduction in, and arrest of, transcriptional activity in target genes. In antagonizing

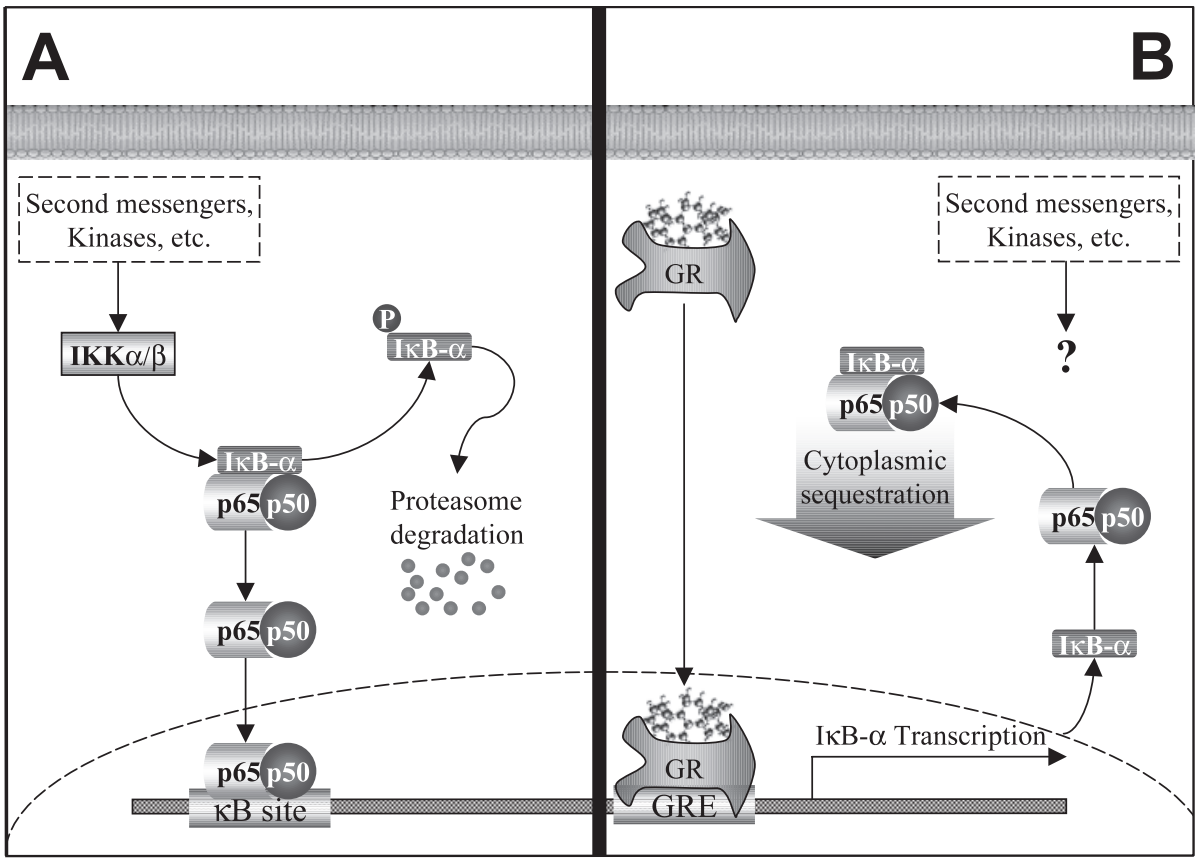

Figure 1 Induction of IκB by GCs. Activation results in phosphorylation (P) of IκB, and its dissociation from NF-κB (p50 and p65) translocates to the nucleus where it binds κB sites. GCs induce IκB synthesis, which subsequently binds to and sequesters NF-κB in the cytosol, thus preventing it from translocating to the nucleus.

transcription factors, GR did not modulate the correct assembly of the pre-initiation complex (TFII–DAB complex), hence localizing its effect upstream of the TATA box (Nissen & Yamamoto 2000). Several mechanisms were postulated for GCs antagonism of NF-κB. These included induction of the synthesis of the NF-κB inhibitor, IκB (Auphan *et al.* 1995, Scheinman *et al.* 1995, Thiele *et al.* 2000), a protein–protein interaction model, which proposes that GR repressed NF-κB activation and/or function either by blocking its access to its DNA (-κB) site (Mukaida *et al.* 1994, Ray & Prefontaine 1994, Newton *et al.* 1998), or by forming a complex with NF-κB which loses DNA capacity (De Bosscher *et al.* 1997, Nissen & Yamamoto 2000), and/or by competition with NF-κB for nuclear co-activators (Zhang *et al.* 1997).

Induction of IκB synthesis

The antagonism of NF-κB activity by GCs was described to be via stimulation of the expression of the NF-κB inhibitor, IκB, synthesis (Auphan *et al.* 1995, Scheinman *et al.* 1995). Increased IκB availability would result in the binding to, and sequestration of, NF-κB in the cytosol, thereby reducing NF-κB nuclear translocation, and hence attenuation of NF-κB-driven transcriptional activities (Fig. 1). This was supported by the findings that treatment of phorbol ester (TPA)-stimulated Jurkat cells (Auphan *et al.* 1995), TNF-stimulated HeLa cells (Scheinman *et al.* 1995), vascular epithelial cells of Crohn's disease patients (Thiele *et al.* 2000), lipopolysaccharide-stimulated macrophages (Crinelli *et al.* 2000), and brain cells (Quan *et al.* 2000) with the GCs dexamethasone (DEX)

or prednisone (Pred) resulted in a concentration-dependent increase in IκB synthesis. Induction of IκB synthesis by GCs (assessed by gel shift and nuclear run-on transcription assays) did not affect IκB phosphorylation and subsequent degradation (Scheinman *et al.* 1995), thus constituting a negative feedback loop whereby increased IκB availability (mediated by GCs) resulted in profound inhibition of NF-κB translocation and activity (Auphan *et al.* 1995, Scheinman *et al.* 1995) (Fig. 1). In addition to its cytosolic site of action, IκB was shown to act at the nuclear level, where it complexed with and dissociated NF-κB from the κB DNA binding sites (Auphan *et al.* 1995, Scheinman *et al.* 1995).

Whereas some reports favored the induction of IκB synthesis by GCs as the mechanism by which GCs antagonized NF-κB activity, other reports failed to establish any link between the induction of IκB synthesis by GCs (if any) and subsequent antagonism of NF-κB by GCs. This was highlighted by the findings that GCs did not stimulate (Kleinert *et al.* 1996) or, according to other reports, decreased IκB synthesis, as was shown in TNFα-activated endothelial cells (De Bosscher *et al.* 1997) and IL-1β-stimulated epithelial cells (Newton *et al.* 1998). Heck *et al.* (1997), using a series of GR mutants, showed that mutants defective in IκB synthesis still antagonized NF-κB. In the same report, using a number of synthetic GCs analogs, it was shown that induction of IκB synthesis by GCs did not lead to repression of NF-κB activity (Heck *et al.* 1997).

In addition, the antagonism of NF-κB by GCs was shown to be independent of IκB induction (Heck *et al.* 1997, De Bosscher *et al.* 2000*b*, Goppelt-Struebe *et al.* 2000). This was based on the findings that in spite of stimulation of IκB synthesis by GCs, increased IκB availability did not affect (De Bosscher *et al.* 1997, 2000*b*) or only partially affected (Crinelli *et al.* 2000) the effects of GCs, and that the effects of GCs were resistant to cycloheximide treatment (De Bosscher *et al.* 1997), thereby arguing against *de novo* induction of an NF-κB inhibitor protein as a potential mechanism by which GCs antagonized NF-κB. Collectively, this ruled out the possibility of induction of *de novo* IκB or a GCs-mediated stabilization of cytosolic NF-κB association with IκB (Scheinman *et al.* 1995) as mechanisms by which GCs repress the transcription of cytokine genes.

In the light of arguments in favor of or against induction of IκB synthesis as the mechanism by which GCs antagonized NF-κB, it appears that stimulation of IκB synthesis and thus antagonism of NF-κB activity by GCs is either an independent event (Bourke & Moynagh 1999, Goppelt-Struebe *et al.* 2000), and/or is cell type specific (Costas *et al.* 2000, De Bosscher *et al.* 2000*b*). However, the latter mechanism is questioned as contradictory effects of GCs on IκB synthesis were observed in the same tissue and cell types. This was exemplified by the reported capacity, according to some reports, of GCs to induce IκB synthesis in brain cells (Quan *et al.* 2000) and in L929 cells (Costas *et al.* 2000), but is in sharp disagreement with other reports which showed that GCs did not affect IκB levels in brain cells (Bourke & Moynagh 1999) or in L929 cells (De Bosscher *et al.* 1997). This prompted the speculation that the effect of GCs on IκB synthesis and subsequently on NF-κB synthesis may be highly cell specific (De Bosscher *et al.* 2000*b*), or due to specific activation signals, and questioned whether stimulation of IκB synthesis by GCs is required or is sufficient to repress NF-κB activity (Heck *et al.* 1997).

Protein–protein interaction

GCs antagonized NF-κB through a protein–protein interaction between the hormone-activated GR and NF-κB subunits. The anti-proliferative effect of GCs was proposed to result from binding of GR to a critical site within NF-κB either prior to DNA binding (Simple Model), or following association of NF-κB with κB DNA binding site (Composite Model). Although association of the GR with DNA (nGRE) was not obligatory, it could not be ruled out.

The simple model

In the simple model, GR binds NF-κB forming a GR–NF-κB complex which does not bind DNA, illustrated in the capacity of the GR to bind to NF-κB (Adcock *et al.* 1995, Kleinert *et al.* 1996, De Bosscher *et al.* 1997), thereby abolishing its capacity to bind κB DNA sites (Fig. 2). In exerting its effect, GR did not alter the nuclear translocation (Adcock *et al.* 1995), or inhibit the synthesis of NF-κB (Newton *et al.* 1998, Kleinert *et al.* 1996), but rather acted by interfering with DNA binding through

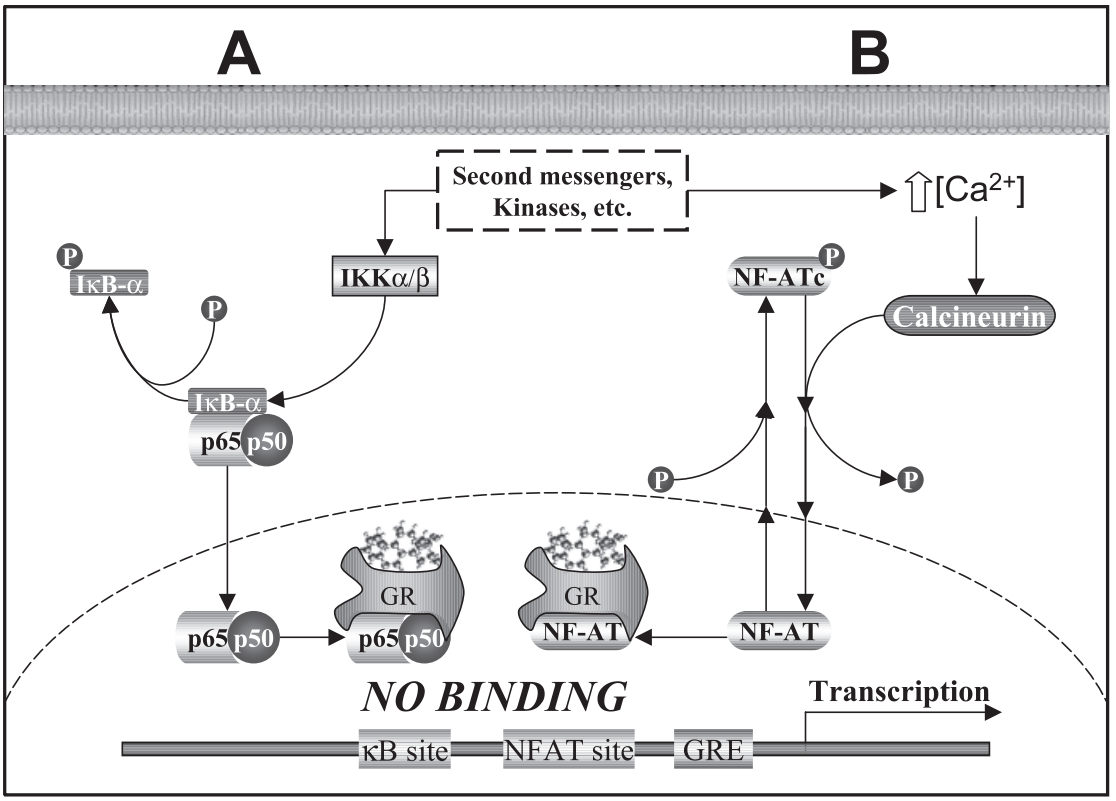

Figure 2 The Simple Model. In the simple model, hormone-activated GR did not affect the availability or translocation of NF-κB or its function, but rather it binds to NF-κB in the nucleus and/or cytosol, thereby forming a complex which failed to bind to DNA.

reciprocal masking of a specific domain within the GR and NF-κB (Fig. 2). This did not result in a competitive displacement of previously DNA-bound NF-κB, but was associated with blockade of the binding of and transactivation by NF-κB (Mukaida *et al.* 1994, Adcock *et al.* 1995, Steer *et al.* 2000), associated with disruption of the interaction of the p65 subunit of NF-κB with basal transcription factor (De Bosscher *et al.* 2000*a*). This was evidenced by protein cross-linking and co-immunoprecipitation of both GR and NF-κB (Ray & Prefontaine 1994), and by the reversal of the GCs effect by over-expression of the NF-κB p65 subunit (Ray & Prefontaine 1994). Furthermore, it was specific for GR since other steroid/thyroid receptors failed to bind to and affect NF-κB transactivation (Caldenhoven *et al.* 1995). Although not tested for NF-κB, the capacity of the GR to inhibit NF-κB binding may, in principle, have resulted from suppression of a key signaling pathway involved in its activation, analogous to that described for the repression of AP-1 activity by GCs. Here, GCs inhibition of AP-1 activity was shown to be the result of proximal inhibition of c-Jun NH2-terminal kinase (JNK), a key mediator of AP-1 activation (Gonzalez *et al.* 2000). It is also plausible that the repression of NF-κB binding by GCs was the consequence of earlier antagonism of the binding and/or activation of other transcription factors, described as being required for efficient NF-κB binding to its putative DNA site (Casolaro *et al.* 1995, Chen *et al.* 2000).

The composite model

Whereas the simple model proposes that GR antagonized NF-κB by preventing its binding to DNA (κB sites) through formation of an inactive GR–NF-κB complex, the composite model stipulates that GR antagonized NF-κB through direct association with NF-κB without altering its DNA binding capability (Fig. 3). This reportedly

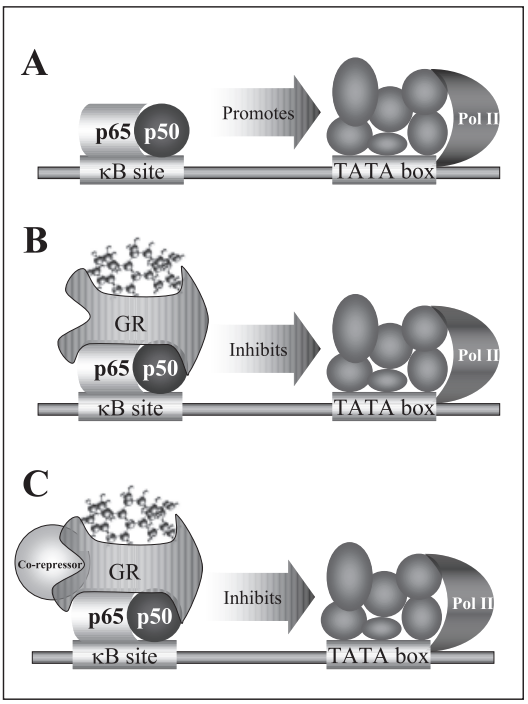

Figure 3 The Composite Model. In the composite model, ligand-activated GR did not bind DNA, but it associated with NF-κB bound at the κB DNA site, leading to inhibition of downstream transcriptional activities, without affecting the correct assembly of the pre-initiating complex at the TATA box. GR may act directly (B), or may require the participation of corepressor(s) (C). Pol II, RNA polymerase II.

involved direct complexing between the two factors involving specific domains within NF-κB and GR. Accordingly, GR did not need to bind DNA, or dissociate NF-κB from DNA binding (Hart *et al.* 2000, Nissen & Yamamoto 2000), but rather acted by associating with NF-κB bound to its putative DNA site, thereby repressing its activity (De Bosscher *et al.* 1997) (Fig. 3). This was evidenced by the capacity of GR to associate with the trans-activating domain of the p65 (RelA) subunit of NF-κB (De Bosscher *et al.* 2000*b*, Nissen & Yamamoto 2000), which, in turn, destabilized the interaction of basal transcription factors (TFII-D) with the TATA binding domain (TBD) (Fig. 3). However, it remains to be seen whether the association of GR with the transcription factor is sufficient to repress the transcriptional activity of the latter or, in addition, requires the participation of a corepressor as was suggested by Nissen and Yamamoto (2000).

The competition model

Insofar as coactivator proteins, including CREB binding protein (CBP), p300, steroid receptor coactivator (SRC)-1, and histone acetyltransferase (HAT) were described as stimulating the activity of NF-κB (Freedman 1999), and as GRs were shown to antagonize NF-κB, it was suggested that GR acted, at least in part, by competing with NF-κB for nuclear coactivators (Kamei *et al.* 1996, Aarnisalo *et al.* 1998) (Fig. 4). In support of this hypothesis were the findings that CBP augmented GR suppressive effects (Kino *et al.* 1999), enhanced the association of GR with, and hence suppressed, NF-κB activity (McKay & Cidlowski 2000, Sheppard *et al.* 1998), and that over-expression of CBP abrogated GR-mediated repression of NF-κB activity (Sheppard *et al.* 1998). GCs down-regulated mRNA and protein accumulation of SRC-1, a key adaptor coactivator, thereby providing for an autoregulatory loop of GCs action (Kurihara *et al.* 2000). Insofar as coactivators, including SRC-1 (Na *et al.* 1998, Sheppard *et al.* 1998) and CBP (Aarnisalo *et al.* 1998) were described as an integral link between basal transcription factors and other transcription factors, including GR and NF-κB (Na *et al.* 1998, Sheppard *et al.* 1998), competition for a limited amount of nuclear coactivators between GR and other induced transcription factors, at least in part, antagonized transcription factors (Fig. 4).

Other reports argued against competition for nuclear coactivator(s) as a mechanism by which GR antagonized transcription factor. For example, GR interacted directly with and inhibited NF-κB activity independently of CBP levels in the cell (De Bosscher *et al.* 2000*b*). While GR inhibited NF-κB binding and activity (Adcock *et al.* 1995, Steer *et al.* 2000), NF-κB DNA binding capacity was not affected by the level of nuclear co-activators, since increased NF-κB levels were seen in the face of overall reduction in CREB binding (Steer *et al.* 2000). Collectively, this questioned whether reduced CREB and other nuclear coactivator functions and GR repression of NF-κB were related. Furthermore, the transactivation and transrepression function of the GR were shown to be separate entities (Heck *et al.* 1997, Belvisi *et al.* 2001), and the requirement for a direct association with specific NF-κB subunits on overall GR function (activation or repression), without necessarily involving an adaptor or competing for a

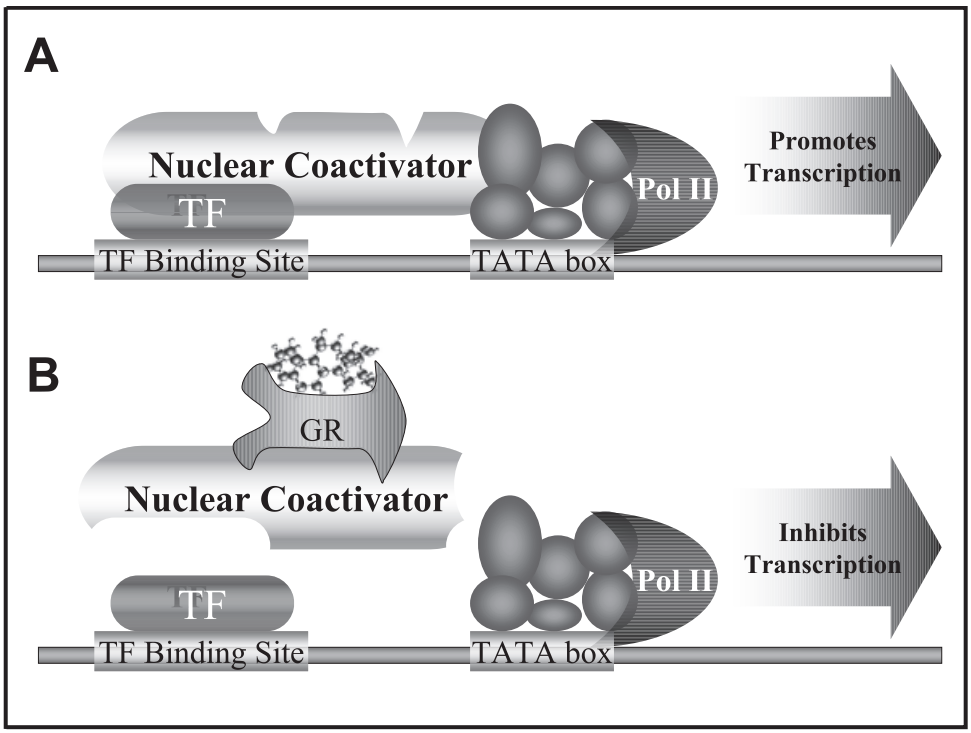

Figure 4 The Competition Model. Hormone-activated GR, by binding to the nuclear coactivator, competes with NF-κB for nuclear coactivators, thereby breaking the link between the transcription factor (TF) and the pre-initiation complex, thus repressing transcription.

nuclear coactivator, are well documented (Pearce *et al.* 1998). Additional studies are required to confirm, or alternatively rule out, competition for nuclear coactivator(s) as a mechanism by which GRs antagonize transcription factors.

Conclusion

During the last two decades significant advances have been made towards understanding the precise mode of action of the GCs, and it now appears to be multi-faceted, affecting both transcriptional and post-transcriptional events. In view of the cooperation between transcription factors in driving optimal transcriptional activation, it remains to be determined whether the effect of GCs on antagonizing NF-κB is a direct event or, alternatively, a consequence of an earlier antagonism of another factor in the activation cascade (Chen *et al.* 2000). The multitude of conclusions drawn from the literature indicate that GCs most likely affect several transcriptional events, as a single mechanism could not apply to all cell types and stimulation conditions. A thorough understanding of the mode of action of the GCs is of paramount importance in better management of GCs toxicity, and in the development of a future immunosuppressive regimen.

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