Molecular mechanisms of glucocorticoid antiproliferative effects: antagonism of transcription factor activity by glucocorticoid receptor

Wassim Y. Almawi* and Ohannes K. Melemedjian†

*Department of Medical Biochemistry, Arabian Gulf University, Manama, Bahrain; and
†Department of Biology, American University of Beirut, Lebanon

Abstract: Glucocorticoids (GCs) exert their anti-inflammatory and immunosuppressive effects by inhibiting the expression of cytokines and adhesion molecules. The molecular basis of GC action lies in their capacity to diffuse through the cell membrane and bind their cytosolic GC receptor (GR), which subsequently undergoes nuclear translocation and modulates transcriptional activation through association with promoter elements, GC response elements (GRE). GR also antagonized the activity of transcription factors, including NF-κB, NF-AT, and AP-1, through direct and indirect mechanisms. GCs induced the gene transcription and protein synthesis of the NF-κB inhibitor, IκB. Activated GR antagonized transcription factor activity through protein:protein interaction. This involved complexing with and inhibition of transcription factor binding to DNA (simple model), association with factor bound at its DNA site (composite model), and/or through interaction of GRE-bound GR with DNA-bound transcription factor (transmodulation model). Finally, GR competed with transcription factors for nuclear coactivators (competition model), including CBP and p300. Remarkably, GR did not affect the assembly of the preinitiation complex but acted proximally in inhibiting transcription factor activity and thus transcriptional initiation. *J. Leukoc. Biol.* 71: 9–15; 2002.

Key Words: *GRE · NF-κB · NF-AT · AP-1 · transactivation · transrepression*

INTRODUCTION

Although glucocorticoids (GCs) are successfully used as potent immunosuppressive and antiproliferative drugs in treating disorders of heightened immunity [1], the molecular mechanism underlying their effects remains not completely understood. It is well appreciated that their mode of action is multifactorial, and evidence of blockade of T-cell immunity at multiple stages by GCs is documented [1]. The most significant mechanism of action of GCs lies in their capacity to block cytokine production [1, 2] and for some cytokines, signaling through their high-affinity receptor complex [3, 4]. Originally, it was postulated that this inhibition required a prerequisite, direct interaction of the activated GC-GC receptor (GR) complex with DNA sites compatible with the GC response element (GRE) motif. These sites are located in variable copy numbers and at variable distances from the TATA box in the promoter region of GC-responsive genes including cytokine genes. Recent evidence suggested that GCs modulate transcription through antagonism of transcription factors required to drive optimal cytokine transcription, including activated protein-1 (AP-1) complex, NF-AT, and nuclear factor-κB (NF-κB). This review focuses on the effect of GCs on transcription-factor activity. For discussion about other aspects of GC-antiproliferative effects, the reader is referred to reviews published elsewhere [1, 5].

MODE OF ACTION OF GCs

Signaling through the antigen-specific T-cell receptor (TcR)/CD3 complex (signal 1) in conjunction with noncognate costimulatory signals, including CTLA-4 and its related homologue CD28, and cytokine receptors (signal 2) results in elevation of intracellular calcium and the induction of calmodulin-regulated kinases, which include the serine-threonine phosphatase calcineurin [6] and activation and translocation of protein kinase C (PKC) from cytosolic to membrane-bound compartments where it expresses its enzymatically active conformation (**Fig. 1**). This, in turn, induced the activation and nuclear translocation of NF-AT (induced by calcineurin) and NF-κB (stimulated by PKC and other signaling pathways), where they bind the interleukin (IL)-2 enhancer and stimulate IL-2 transcription [7] (Fig. 1). An orderly expression of cytokine genes and their high-affinity receptors ensues, followed by induction of T-cell proliferation [8].

GCs antiproliferative effect is largely the result of inhibition of cytokine expression. GCs inhibited the expression of IL-1 [9], IL-2 [10, 11], IL-3 [12], IL-4 [13], IL-5 [2], IL-6 [9], IL-8 [14], IL-11 [15], IL-12 [16, 17], IL-16 [18], interferon-γ (IFN-γ) [19], tumor necrosis factor-α (TNF-α) [20], and the

Correspondence: Dr. Wassim Y. Almawi, Department of Medical Biochemistry, College of Medicine & Medical Sciences, Arabian Gulf University, P.O. Box 22979, Manama, Bahrain. E-mail: wassim@agu.edu.bh

Received October 2, 2001; accepted October 15, 2001.

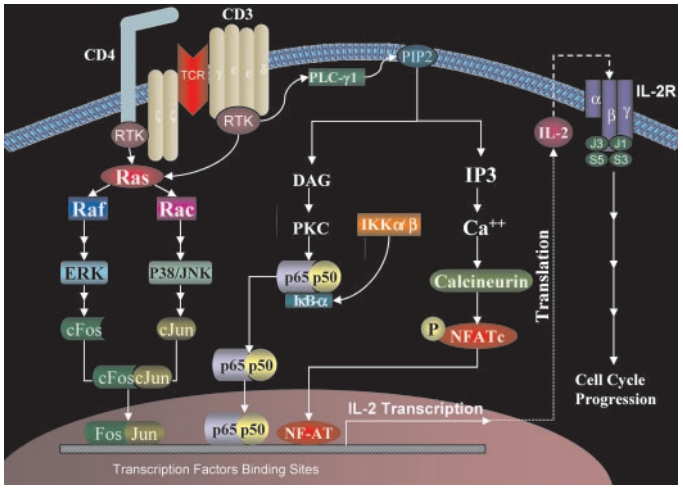

Fig. 1. T-cell activation cascade. T-cell activation through the multimeric T-cell receptor-CD3 complex results in the activation of several intracellular signaling pathways leading to phosphorylation or dephosphorylation of cytoplasmic target molecules. These converge at provision and nuclear translocation of transcription factors NF-κB, NF-AT, and the AP-1 complex, which subsequently bind their specific upstream DNA sites, hence facilitating RNA polymerase II-directed activity.

colony-stimulating factors (CSF), macrophage (M)-CSF [21], granulocyte (G)-CSF [22], CSF-1 [23], and granulocyte-macrophage (GM)-CSF [14]. Inhibition of cytokine expression was specific for the GCs because non-GC steroids failed to affect cytokine synthesis [24] or T-cell proliferation [25] and as the GR antagonist, RU-486 abrogated GC effects [4, 11, 16]. Inhibition of cytokine expression by GCs involved transcriptional and posttranscriptional events (see below) and resulted in a significant reduction in cytokine secretion and a profound inhibition of T-cell-effector function. Furthermore, recombinant cytokines abrogated GC effects, including apoptosis [26], inhibition of cytokine expression [15], and suppression of T-cell proliferation [10]. Kinetic differences exist between different GCs in inhibiting cytokine expression and/or T-cell activation, depending on the GC itself, stimulus used, and activation status [25].

REPRESSION OF CYTOKINE GENE EXPRESSION BY GC DIRECT MECHANISMS

Because of their lipophylic nature and low molecular weight, GCs passively diffuse through the plasma membrane where they bind their intracellular GR, which functions as a ligand-activated, dual transcription factor. Depending on the target gene, hormone-activated GRs may stimulate (transactivation) or inhibit (transrepression) gene transcription. The former is exemplified by the demonstrated capacity of GCs to stimulate the production of the NF-κB inhibitor, IκB [27, 28], and the latter is exemplified by the demonstrated effect of GCs in suppressing the expression of IL-2 and other cytokine genes [1].

In the cytoplasm, GRs exist as an inactive complex with two molecules of heat-shock protein (HSP-90) and other cytosolic proteins [29]. Upon binding its cognate ligand (GCs), GR undergoes conformational changes, dissociates from HSP-90 binding, and subsequently translocates to the nucleus where it transiently associates with HSP-56. GR, in turn, dissociates from HSP-56 and binds as a dimer to conserved palindromic DNA sequences, the GREs, which comprise two boxes spaced by three variable nucleotides (GGTACAnnnTGTTCT), each box binding one of the two GR zinc fingers [30]. GREs are located in variable copy numbers and are found at variable distances from the TATA box in the promoter region of GC responsive genes, including cytokine genes.

The GR, a member of the steroid superfamily (which includes steroid, thyroid hormone, vitamin D, and retinoic acid receptors [31]), consists of three domains: a hormone-binding domain, a highly conserved DNA-binding domain, and an N-terminal region [31]. An intact GRE site [32] and DNA binding domain within the GR [33] are required for efficient transcriptional modulation by GCs. Binding of activated GRs complex to GRE-DNA elements results in downstream inhibition of cytokine gene expression in a cis-acting (steric hindrance) [34] or, alternatively, trans-acting fashion, the latter involving induction of the synthesis of specific inhibitor(s). According to the former, GR-GRE binding is viewed as that of a masking effect, where GR binds to sites overlapping the binding sites of basal [35] and induced [36] transcription factors, resulting in steric interference with the binding of transcription/enhancing factors (NF-κB, NF-AT) to their putative DNA sites. The latter involves expression of a specific GC-induced inhibitor of T-cell activation [37], including the NF-κB antagonist IκB [27]. It should be noted here that although evidence supporting both possibilities is documented, conclusions drawn must be viewed in the context of the cell type and gene studied.

INDIRECT MECHANISMS: ANTAGONISM OF TRANSCRIPTION FACTORS

Although the GR-GRE interaction model provided for a framework to elucidate the molecular mechanism of GC modulation of gene activation, recent evidence demonstrated that GCs acted transcriptionally by antagonizing transcription factor activation or function. This was evidenced by the demonstrated capacity of GRs to repress transcription of cytokine genes through interference with the nuclear translocation and/or function of the transcription factors AP-1 (dimer of c-Fos and c-Jun) [2, 38], NF-κB [34, 39, 40], and NF-AT [13, 33, 41]. Reduction in transcription factor availability and function subsequently translated into reduction and eventually cessation of transcription in target genes. It is noteworthy that GR did not interfere with the assembly of the preinitiation complex (including association of RNA polymerase II with TFII complex of nuclear proteins), thereby localizing its effect at DNA sites upstream of the TATA box [42]. Several mechanisms were postulated for GC effects, including induction of production of NF-κB inhibitor, IκB [27, 28, 43]; protein:protein interaction, where GR prevented transcription-factor binding to DNA sites [44, 45], repressed the activity of the transcription factor without affecting its binding onto DNA [34, 42], or associated with the factor, thereby becoming functionally (repressive) active; and competition with transcription factor for nuclear

coactivators [46, 47]. Elucidation of the exact mechanism of action of the GCs and the scope of interaction of the activated GR with AP-1, NF-κB, NF-AT, and other transcription factors are of paramount importance toward development of a more efficacious anti-inflammatory and immunosuppressive regimen.

INDUCTION OF IκB SYNTHESIS

NF-κB, a member of the mammalian rel gene family, which comprised p105/p50, p100/p52, p65 (RelA), RelB, and others [48], 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 (**Fig. 2**). IκB, a member of a large family of inhibitory molecules that comprised IκBα, IκBβ, IκBε, and IκBγ [49], masked the nuclear localization signal of NF-κB, resulting in its retention in the cytoplasm. Activation by extracellular signals supposedly induced the 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 [49] (Fig. 2). 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 [48, 49], including cytokine and IκB genes [28, 50]. Persistent NF-κB activation, in turn, leads to increased IκB synthesis and sequesters cytosolic NF-κB, thereby attenuating its activity [48, 50].

Several studies documented the capacity of GCs to induce IκB synthesis [27, 28]. GC treatment of phorbol ester 12-*O*-tetradecanoylphorbol 13-acetate (TPA)-stimulated Jurkat cells [27], TNF-stimulated HeLa cells [28], vascular epithelial cells of Crohn's disease patients [43], lipopolysaccharide (LPS)-stimulated macrophages [20], and brain cells [51] resulted in a concentration-dependent induction of IκB synthesis. According to these studies, GC treatment induced IκB synthesis (assessed by gel shift and nuclear run-on transcription assays) without affecting its phosphorylation and subsequent degradation [28]. This constituted a negative feedback loop, whereby IκB binds to and sequesters NF-κB in the cytosol and prevents it from translocating to the nucleus [27, 28]. It was even proposed that IκB acted at the level of nucleus, binding and removing NF-κB from binding κB sites [27, 28], although the universality of this finding remains to be determined.

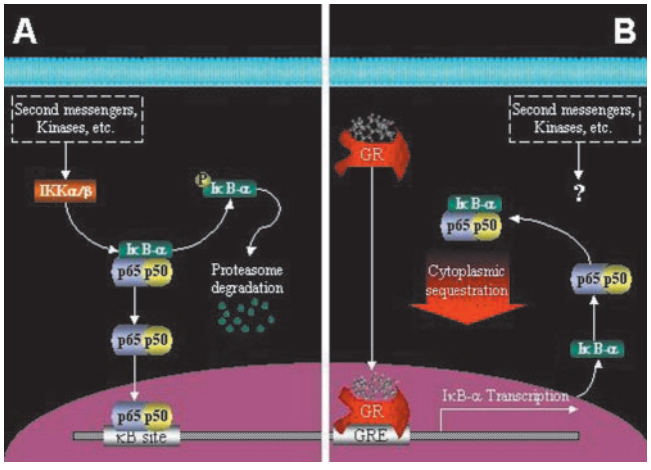

Fig. 2. Induction of IκB synthesis by GCs. Cellular activation results in phosphorylation of IκB (mediated by the IκB kinases IKKα and β) and its dissociation from NF-κB binding and degradation by proteasomes. NF-κB (p50 and p65) then translocates to the nucleus where it binds κB sites. By inducing IκB synthesis, GC provides for increasing IκB availability, which subsequently binds to and sequesters NF-κB in the cytosol, hence preventing it from translocating to the nucleus.

Several reports disputed induction of IκB synthesis as the mechanism by which GCs repressed gene activation, which was based along several lines of reasoning. First, it was based on the finding that in many cell types, GC treatment did not stimulate [52] or actually decreased IκB synthesis, as was shown for TNF-α-activated endothelial cells [34] and IL-1β-stimulated epithelial cells [45]. GR mutants, which did not enhance IκB synthesis, repressed NF-κB activity [40]. Conversely, induction of IκB synthesis by synthetic GC analogues failed to repress NF-κB activity [40]. Furthermore, GC-mediated down-regulation of NF-κB was described to be independent of IκB induction [39, 40, 53], because despite clear induction of IκB synthesis by GCs, increased IκB synthesis did not [34, 39], or only partially affected [20], GC anti-inflammatory and immunosuppressive effects and because GC effects were resistant to cycloheximide treatment [34]. This effectively argued against induction of de novo IκB synthesis or a GC-mediated stabilization of cytosolic NF-κB association with IκB [28] as potential mechanisms by which GCs repress transcriptional of cytokine genes.

This indicated that GC induction of IκB synthesis and thus antagonism of NF-κB activity is an independent event [53, 54] or is cell-type specific [39, 55]. However, the latter is questioned, because contradicting effects of GCs on IκB synthesis were observed in the same tissue and cell types. This was exemplified by the demonstrated capacity of GCs according to some studies to induce IκB synthesis in brain cells [51] and in L929 cells [55] but in sharp disagreement with other studies, which showed that GCs did not affect IκB levels in brain cells [54] or in L929 cells [34]. This prompted the speculation that GC effects on IκB synthesis and subsequently on NF-κB synthesis may be highly cell-specific [39] or a result of specific activation signals, and this questioned whether stimulation of IκB synthesis by GCs is even required or sufficient to repress NF-κB activity [40].

PROTEIN:PROTEIN INTERACTION MODEL

A second mechanism by which GCs interacted with and antagonized transcription factors involved a protein:protein crosstalk between GR and transcription factors, including NF-AT, NF-κB, and AP-1. According to this model, proposed and increasingly being adopted by several investigators, the GC antiproliferative effect is viewed as the result of binding GR to a critical site within the transcription factor prior to DNA binding (simple model) or following association with the DNA-binding site of the transcription factor (composite model). Although direct association of the GR with DNA, for example, through binding negative GREs, was found not to be obligatory, it was not ruled out completely. Protein:protein interaction

between hormone-activated GR and transcription factor resulted in interference with the functional capacity of the transcription factor to stimulate transcription.

THE "SIMPLE" MODEL

The simple model postulates that GR binds the transcription factor, thereby forming a complex that does not bind DNA (**Fig. 3**). Evidence supporting the simple model was illustrated by the capacity of GRs to bind to NF-AT [13], NF-κB [34, 52, 56], and AP-1 [56, 57], thereby abolishing their capacity to bind DNA. In exerting its effect, GR did not alter the nuclear translocation [13, 56] or inhibit the synthesis of the transcription factor [45, 52] but acted by interfering with DNA binding through reciprocal masking of a specific domain within the GR and the transcription factor (Fig. 3) [38]. This did not result in a competitive displacement of transcription factor bound to DNA but was associated with antagonism of binding [56, 58], evidenced by the coimmunoprecipitation of GR and nuclear transcription factor [13, 44] and by the reversal of the GC effect by overexpression of transcription factor [44], although the universality of the latter conclusion could not be confirmed. From suppression of a key signaling pathway involved in transcription-factor activation, the capacity of the GR to inhibit transcription-factor binding may have, in principle, exemplified a key mediator of AP-1 activation [59] or possibly as a consequence of earlier antagonism of other transcription factors [13], evidenced by the demonstrated capacity of the GCs to antagonize AP-1 binding through inhibition of c-Jun NH_2-terminal kinase (JNK).

THE "COMPOSITE" MODEL

GR antagonized transcription-factor activity through direct association with the factor without altering its DNA-binding capability in a mechanism involving specific domains within the transcription factor and GR. Accordingly, GR exerted its effect, not by binding DNA or dissociating the transcription factor from DNA binding [42, 60] but by associating with the factor bound at its putative DNA site, thereby repressing its activity [34]. This was evidenced by the capacity of GR to associate with the transactivating domain of the p65 (RelA) subunit of NF-κB [39, 42], which, in turn, destabilized the interaction of basal transcription factors (TFIID) with the TATA-binding domain (TBD). In addition to NF-κB, GR also inhibited AP-1 activity without altering the binding of AP-1 (Fos-Jun) or itself binding to DNA [61], as evidenced by the capacity of GR mutants lacking the DNA-binding domain to bind to and inhibit AP-1 activity [57, 62]. Collectively, this indicated that GR acted by forming a complex with the transcription factor at the binding site of the latter, which, in turn, attenuates and eventually terminates transcription (**Fig. 4**). 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 (Fig. 4) [42].

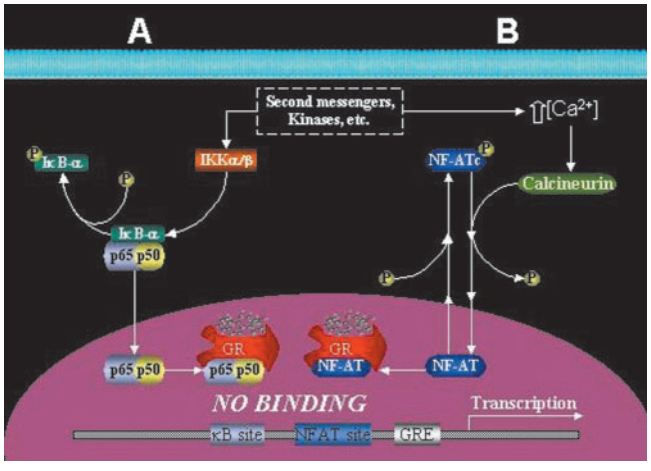

Fig. 3. The "simple model" of protein:protein interaction. According to the simple model, the hormone-activated GR did not affect the availability or translocation of transcription factors (exemplified by NF-AT and NF-κB) but rather acted more distally in binding to the transcription factor in the nucleus and/or cytosol, thereby forming a complex, which failed to bind to DNA.

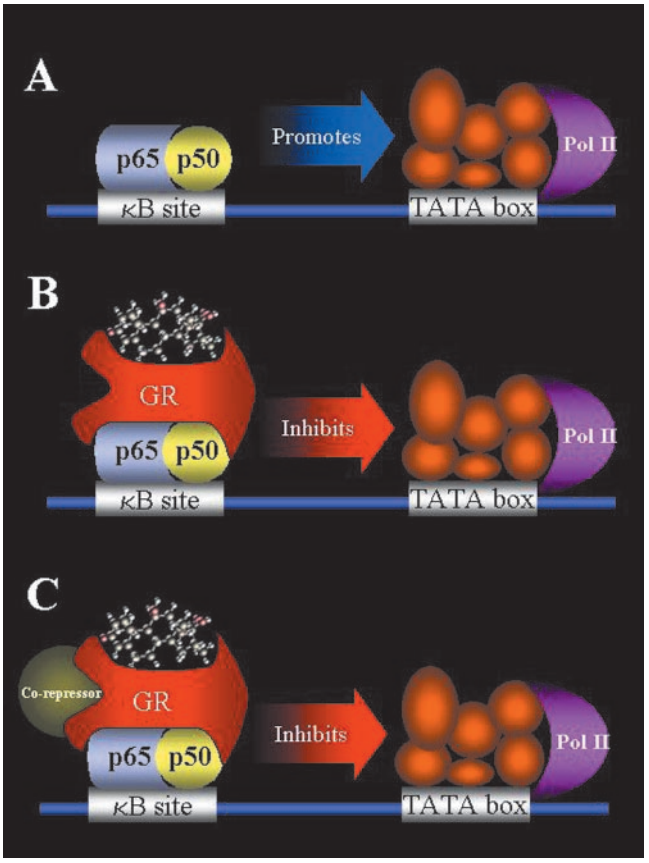

Fig. 4. The "composite model" of protein:protein interaction. In the composite model, ligand-activated GR did not bind DNA but associated with transcription factor bound at its respective DNA site, as shown for NF-κB bound to the κB site. This association inhibited downstream transcriptional activities, without affecting the correct assembly of the preinitiating complex at the TATA box. GR may act directly (B) or may require the participation of corepressor(s) (C).

TRANSMODULATION MODEL

The transmodulation model postulates that interaction of GRE-bound GR with DNA-bound transcription factor, which oc-

curred through a direct protein:protein binding, conferred a functional attribute on GR and resulted in profound inhibition of transcription. This facilitated direction of ligand-activated GR to its DNA sites (GREs) [63], stabilized the interaction of GR with GRE-like DNA sites, or induced conformational changes in the topology of DNA, which prevented initiation of proper transcription [64]. In support of this scheme were the findings that AP-1 [41, 65, 66], NF-AT [13, 33, 41], and ANF-1 and ANF-2 [66] facilitated transcriptional repression by GR. It is noteworthy that GCs did not block the expression of NF-κB, AP-1, AP-3, Oct-1, and NF-AT [13, 33, 67], and evidence of the formation of a complex of these factors with GR (assessed by double immunoprecipitation) was shown.

Correct spacing between GRE and transcription factor binding sites was necessary for GR to exert its effect. Interaction of ligand-bound GR with AP-1, where GRE sites were positioned within a few bases from the AP-1-binding site, resulted in repression of AP-1-driven activity [64]. This did not result from occupancy of the AP-1 site or from inhibition of AP-1 binding, because both factors were shown to be clearly bound to their DNA sites, but rather involved a direct protein:protein association of GR with c-Fos and thus repression of AP-1 activity [64]. It was interesting to note that composition of AP-1 influenced GR activity according to this model, because Jun-Fos heterodimers, but not Jun-Jun homodimers, associated with GR [68] and because c-Fos (but not c-Jun) induced the expression of GR [69] and associated with it [64, 70]. Collectively, this suggested that the association of transcription factors with GR, coupled with the close proximity of their DNA-binding sites to GRE sites, prevented correct assembly of the preinitiation complex and hence initiation of proper transcription.

COMPETITION MODEL

Insofar as coactivator proteins, including CREB-binding protein (CBP), p300, steroid receptor coactivator (SRC)-1, and histone acetyltransferase (HAT), were described to stimulate the activity of transcription factors, including AP-1 and NF-κB [71], and as GRs were shown to antagonize transcription factors, it was suggested that GRs acted, at least in part, by competing with nuclear transcription factors for nuclear coactivators (**Fig. 5**) [47, 72]. In support of this were the findings that CBP augmented GR-suppressive effects [73], enhanced the association of GR with CBP, and hence suppressed NF-κB activity [46, 74], and as overexpression of CBP, abrogated GR-mediated repression of NF-κB activity [46]. GCs down-regulated mRNA and protein accumulation of SRC-1, a key "adaptor" coactivator, thereby providing for an autoregulatory loop of GC action [75]. Insofar as coactivators, including SRC-1 [46, 76] and CBP [72], were described as an integral link between basal transcription factors and other transcription factors, including GR and NF-κB [72, 76], competition for a limited amount of nuclear coactivators between GR and other induced transcription factors, at least in part, antagonized transcription factors (Fig. 5).

Other studies argued against competition for nuclear coactivator(s) as a mechanism by which GR antagonized transcription factor. For example, GR interacted directly with AP-1 [39] and NF-κB [39], independently of CBP levels in the cell. Increased AP-1 and NF-κB binding was associated with overall reduced CREB binding [56], thereby questioning whether reduced CREB and other coactivator function and GR repression of transcription factors were related. Indeed, the transactivation and transrepression functions of the GR were shown to be separate entities [40, 77], and the requirement for direct association with specific AP-1 or NF-κB subunits on overall GR function (activation or repression), without necessarily involving an adaptor or competing for a nuclear coactivator, is well-documented [39, 64, 78]. Additional studies are required to confirm or alternatively rule out competition for nuclear coactivator(s) as a mechanism by which GRs antagonize transcription factors.

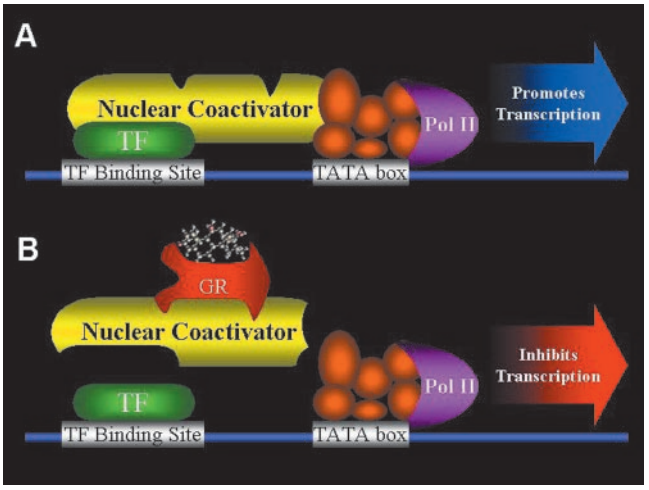

Fig. 5. The "competition model". Coactivator proteins, by associating with transcription factors (TF) bound at their specific DNA sites (TF-binding sites) and the preinitiation complex of basal TFs and RNA polymerase II, facilitate TF effects, thereby promoting transcription. GR, by binding to the nuclear coactivator, competes with TF for the coactivator, thereby breaking the link between TF and the preinitiation complex, thus repressing transcription.

ANTAGONISM OF TRANSCRIPTION FACTORS BY GLUCOCORTICOID-REGULATED GENES

In addition to the above-mentioned mechanisms, recent studies suggested that GCs exerted their effects, at least in part, through induction of specific GC-regulated genes, GILZ (GC-induced leucine zipper) and GITR (GC-induced TNFR family-related) [79, 80]. GILZ, a 137-amino acid leucine-zipper transcription factor, is expressed constitutively in normal cells and up-regulated by GCs [79, 81]. GILZ supposedly mediated a number of GC effects, including modulation of activation-induced cell death [79, 81] and antagonism of transcription factors [82, 83]. GILZ was shown to act by inhibiting NF-κB translocation and hence its binding to the IκB DNA site through a protein:protein interaction [83] and to interact with c-Fos, thereby inhibiting the translocation of AP-1 (dimer of Fos and Jun) and subsequently its DNA binding [82]. Collectively, this suggested that GC antagonism of NF-κB and AP-1 activity results, at least in part, from induction of GILZ.

CONCLUSION

During the last two decades, significant advances have been made toward understanding the precise mode of action of the GCs, and it now appears to be multifaceted, affecting transcriptional and posttranscriptional events. In view of the cooperation between transcription factors in driving optimal transcriptional activation, exemplified by the obligate induction of AP-1 on subsequent NF-AT activation, it remains to be determined whether the GC effect on antagonizing a transcription factor is a direct event or, alternatively, a consequence of an earlier antagonism of another factor in the activation cascade [13]. The many conclusions drawn from the literature indicate that GCs most likely affect several transcriptional events, because 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 GC toxicity and in the development of a future immunosuppressive regimen.

REFERENCES

1. Almawi, W. Y., Hess, D. A., Rieder, M. J. (1998) Multplicity of glucocorticoids action in inhibiting allograft rejection. Cell Transplant. 7, 511–523.
2. Mori, A., Kaminuma, O., Suko, M., Inoue, S., Ohmura, T., Hoshino, A., Asakura, Y., Miyazawa, K., Yokota, T., Okumura, Y., Ito, K., Okudaira, H. (1997) Two distinct pathways of interleukin-5 synthesis in allergen-specific human T-cell clones are suppressed by glucocorticoids. Blood 89, 2891–2900.
3. Almawi, W. Y., Hess, D. A., Rieder, M. J. (1998) Significance of enhanced cytokine receptor expression by glucocorticoids. Blood 92, 3979–3981.
4. Almawi, W. Y., Tamim, H. (2001) Post-transcriptional mechanisms of glucocorticoid anti-proliferative effects. Glucocorticoids inhibit IL-6-induced proliferation of B9 hybridoma cells. Cell Transplant. 10, 161–164.
5. Suthanthiran, M., Strom, T. B. (1995) Immunobiology and immunopharmacology of organ allograft rejection. J. Clin. Immunol. 15, 161–171.
6. Clipstone, N. A., Crabtree, G. R. (1992) Identification of calcineurin as a key signaling enzyme in T lymphocyte activation. Nature 357, 695–697.
7. Blank, V., Kowilsky, P., Israel, A. (1992) NF-κB and related proteins: Rel/dorsal homologies meet ankyrin-like repeats. Trends Biochem. Sci. 17, 135–139.
8. Germaine, R. N. (1994) MHC-dependent antigen processing and peptide presentation: providing ligands for T lymphocyte activation. Cell 76, 287–299.
9. Amano, Y., Lee, S. W., Allison, A. C. (1993) Inhibition by glucocorticoids of the formation of interleukin-1α, interleukin-1β, and interleukin-6: mediation by decreased mRNA stability. Mol. Pharmacol. 43, 176–182.
10. Almawi, W. Y., Lipman, M. L., Stevens, A. C., Zanker, B., Hadro, E. T., Strom, T. B. (1991) Abrogation of glucocorticoid-mediated inhibition of T cell proliferation by the synergistic action of IL-1, IL-6, and IFN-gamma. J. Immunol. 146, 3523–3527.
11. Paliogianni, F., Boumpas, D. T. (1995) Glucocortcoids regulate calcineurin-dependent transactivating pathways for interleukin-2 gene transcription in human T lymphocytes. Transplantation 59, 1333–1339.
12. Culpepper, J., Lee, F. (1987) Glucocorticoids regulation of lymphokine production by murine T lymphocytes. Lymphokine 13, 275–280.
13. Chen, R., Burke, T. F., Cumberland, J. E., Brummet, M., Beck, L. A., Casolaro, V., Georas, S. N. (2000) Glucocorticoids inhibit calcium- and calcineurin-dependent activation of the human IL-4 promoter. J. Immunol. 164, 825–832.
14. Tobler, A., Meier, R., Seitz, M., Dewald, B., Baggiolini, M., Fey, M. F. (1992) Glucocorticoids downregulate gene expression of GM-CSF-NAP-1/IL-8, and IL-6, but not M-CSF, in human fibroblasts. Blood 79, 45–51.
15. Haynesworth, S. E., Baber, M. A., Caplan, A. I. (1996) Cytokine expression by human marrow-derived mesenchymal progenitor cells in vitro: effects of dexamethasone and IL-1α. J. Cell. Physiol. 166, 585–592.
16. Elenkov, I. J., Papanicolaou, D. A., Wilder, R. L., Chrousos, G. P. (1996) Modulatory effects of glucocorticoids and catecholamines on human interleukin-12 and interleukin-10 production: clinical implications. Proc. Assoc. Am. Phys. 108, 374–381.
17. Blotta, M. H., DeKruyff, R. H., Umetsu, D. T. (1997) Corticosteroids inhibit IL-12 production in human monocytes and enhance their capacity to induce IL-4 synthesis in CD4+ lymphocytes. J. Immunol. 158, 5589–5595.
18. Arima, M., Plitt, J., Stellato, C., Bickel, C., Motojima, S., Makino, S., Fukuda, T., Schleimer, R. P. (1999) Expression of interleukin-16 by human epithelial cells. Inhibition by dexamethasone. Am. J. Respir. Cell Mol. Biol. 21, 684–692.
19. Kunicka, J. F., Talle, M. A., Denhardt, G. H., Brown, M., Prince, L. A., Goldstein, G. (1993) Immunosuppression by glucocorticoids: inhibition of production of multiple lymphokines by in vivo administration of dexamethasone. Cell. Immunol. 149, 39–49.
20. Crinelli, R., Antonelli, A., Bianchi, M., Gentilini, L., Scaramucci, S., Magnani, M. (2000) Selective inhibition of NF-κB activation and TNF-α production in macrophages by red blood cell-mediated delivery of dexamethasone. Blood Cells Mol. Dis. 26, 211–222.
21. Campbell, R., Ianches, G., Hamilton, J. A. (1993) Production of macrophage colony-stimulating factor (M-CSF) by human articular cartilage and chondrocytes. Modulation by interleukin-1 and tumor necrosis factor. Biochim. Biophys. Acta 1182, 57–63.
22. Hamilton, J. A., Whitty, G. A., Royston, A. K., Cebon, J., Layton, J. E. (1992) Interleukin-4 suppresses granulocyte colony-stimulating factor and granulocyte-macrophage colony-stimulating factor levels in stimulated human monocytes. Immunology 76, 566–571.
23. Chambers, S. K., Gilmore-Hebert, M., Wang, Y., Rodov, S., Benz Jr., E. J., Kacinski, B. M. (1993) Posttranscriptional regulation of colony-stimulating factor-1 (CSF-1) and CSF-1 receptor gene expression during inhibition of phorbol ester-induced monocytic differentiation by dexamethasone and cyclosporin A: potential involvement of a destabilizing protein. Exp. Hematol. 21, 1328–1334.
24. Boumpas, D. T., Chrousos, G. P., Wilder, R. L., Cupps, T. R., Balow, J. E. (1993) Glucocorticoids therapy for immune-mediated diseases: basic and clinical correlates. Ann. Intern. Med. 119, 1198–1208.
25. Almawi, W. Y., Saouda, M. S., Stevens, A. C., Lipman, M. L., Barth, C. M., Strom, T. B. (1996) Partial mediation of glucocorticoid anti-proliferative effects by lipocortins. J. Immunol. 156, 3523–3529.
26. Mor, F., Cohen, I. R. (1996) IL-2 rescues antigen-specific T cells from radiation or dexamethasone-induced apoptosis. Correlation with induction of Bcl-2. J. Immunol. 156, 515–522.
27. Auphan, N., Didonato, J. A., Rosette, C., Helmberg, A., Karin, M. (1995) Immunosuppression by glucocorticoids: inhibition of NF-κB activity through induction of IκB synthesis. Science 270, 286–289.
28. Scheinman, R., Cogswell, P. C., Lofquist, A., Baldwin, A. S. (1995) Role of transcriptional activation of IκBα in mediation of immunosuppression by glucocorticoids. Science 270, 283–286.
29. Oakley, R. H., Jewell, C. M., Yudt, M. R., Bofetiado, D. M., Cidlowski, J. A. (1999) The dominant negative activity of the human glucocorticoid receptor β isoform. Specificity and mechanisms of action. J. Biol. Chem. 274, 27857–27866.
30. Berg, J. M. (1989) DNA binding specificity of steroid receptors. Cell 57, 1065–1068.
31. Evans, R. M. (1988) The steroid and thyroid hormone receptor superfamily. Science 240, 889–895.
32. Goswami, R., Lacson, R., Yang, E., Sam, R., Unterman, T. (1994) Functional analysis of glucocorticoid and insulin response sequences in the rat insulin-like growth factor-binding protein-1 promoter. Endocrinology 134, 736–743.
33. Northrop, J. P., Crabtree, G. R., Mattila, P. S. (1992) Negative regulation of interleukin 2 transcription by the glucocorticoid receptor. J. Exp. Med. 175, 1235–1245.
34. De Bosscher, K., Leinhard-Schmitz, M., Vanden Berghe, W., Plaisance, S., Fiers, W. (1997) Glucocorticoid-mediated repression of nuclear factor-κB-dependent transcription involves direct interference with transactivation. Proc. Natl. Acad. Sci. USA 94, 13504–13509.
35. Ray, A., Sehgal, P. B. (1992) Cytokines and their receptors: molecular mechanism of interleukin-6 gene repression by glucocorticoids. J. Am. Soc. Nephrol. 2, S214–S221.
36. Mordacq, J. C., Linzer, D. I. H. (1989) Co-localization of elements required for phorbol ester stimulation and glucocorticoid repression of proliferin gene expression. Genes Dev. 3, 760–769.
37. Samuelsson, M. K., Pazirandeh, A., Davani, B., Okret, S. (1999) p57Kip2, a glucocorticoid-induced inhibitor of cell cycle progression in HeLa cells. Mol. Endocrinol. 13, 1811–1822.
38. Vacca, A., Felli, M. P., Farina, A. R., Martinotti, S., Maroder, M., Screpanti, I., Meco, D., Petrangeli, E., Frati, L., Gulino, A. (1992) Glucocorticoid receptor-mediated suppression of the interleukin 2 gene expression through impairment of the cooperativity between nuclear factor of activated T cells and AP-1 enhancer elements. J. Exp. Med. 175, 637–646.
39. De Bosscher, K., Vanden Berghe, W., Vermeulen, L., Plaisance, S., Boone, E., Haegeman, G. (2000) Glucocorticoids repress NF-κB-driven

genes by disturbing the interaction of p65 with the basal transcription machinery, irrespective of coactivator levels in the cell. Proc. Natl. Acad. Sci. USA 97, 3919–3924.
40. Heck, S., Bender, K., Kullmann, M., Gottlicher, M., Herrlich, P., Cato, A. C. B. (1997) IκBβ-independent downregulation of NF-κB activity by glucocorticoid receptor. EMBO J. 16, 4698–4707.
41. Paliogianni, F., Raptis, A., Ahuja, S. S., Najjar, S. M., Boumpas, D. T. (1993) Negative regulation of human interleukin 2 (IL-2) gene by glucocorticoids through interference with nuclear transcription factors AP-1 and NF-AT. J. Clin. Investig. 91, 1481–1489.
42. Nissen, R. M., Yamamoto, K. R. (2000) The glucocorticoid receptor inhibits NFκB by interfering with serine-2 phosphorylation of the mRNA polymerase II carboxy-terminal domain. Genes Dev. 14, 2314–2329.
43. Thiele, K., Bierhaus, A., Autschbach, A., Hafmann, M., Stremmel, W., Thiele, H., Ziegler, R., Nawroth, P. P. (1999) Cell specific effects of glucocorticoid treatment on the NF-κBp65/IκB system in patients with Crohn's disease. Gut 45, 693–704.
44. Ray, A., Prefontaine, K. E. (1994) Physical association and functional antagonism between the p65 subunit of transcription factor NF-κB and the glucocorticoids receptor. Proc. Natl. Acad. Sci. USA 91, 752–756.
45. Newton, R., Hart, L. A., Stevens, D. A., Bergmann, M., Donnelly, L. E., Adcock, I. M., Barnes, P. J. (1998) Effect of dexamethasone on interleukin-1β-(IL-1β)-induced nuclear factor-κB (NF-κB) and κB-dependent transcription in epithelial cells. Eur. J. Biochem. 254, 81–89.
46. Sheppard, K. A., Phelps, K. M., Williams, A. J., Thanos, D., Glass, C. K., Rosenfeld, M. G., Gerritsen, M. E., Collins, T. (1998) Nuclear integration of glucocorticoids receptor and nuclear factor-κB signaling by CREB-binding protein and steroid receptor coactivator-1. J. Biol. Chem. 273, 29291–29294.
47. Kamei, Y., Xu, L., Heinzel, T., Torchia, J., Kurokawa, R., Gloss, B., Lin, S. C., Hayman, R. A., Rose, D. W., Glass, C. K., Rosenfeld, M. G. (1996) A CBP integrator complex mediates transcriptional activation and AP-1 inhibition by nuclear receptors. Cell 85, 403–414.
48. Baeuerle, P. A., Baltimore, D. (1996) NF-κB: ten years after. Cell 87, 13–20.
49. Ghosh, S., May, M. J., Kopp, E. B. (1998) NF-κB and Rel proteins: evolutionary conserved mediators of immune responses. Annu. Rev. Immunol. 16, 225–260.
50. Beg, A. A., Baldwin, A. S. (1993) The IκB proteins: multifunctional regulators of Rel/NF-κB transcription factors. Genes Dev. 7, 2064–2070.
51. Quan, N., He, L., Lai, W., Shen, T., Herkenham, M. (2000) Induction of IκBα mRNA expression in the brain by glucocorticoids: a negative feedback mechanism for immune-to-brain signaling. J. Neurosci. 20, 6473–6477.
52. Kleinert, H., Euchenhofer, C., Ihrig-Biedert, I., Forstermann, U. (1996) Glucocorticoids inhibit the induction of nitric oxide synthase II by down-regulating cytokine-induced activity of transcription factor nuclear factor-κB. J. Pharmacol. Exp. Therapeut. 49, 15–21.
53. Goppelt-Struebe, M., Rehm, M., Schaefers, H. J. (2000) Induction of cyclooxygenase-2 by platelet-derived growth factor (PDGF) and its inhibition by dexamethasone are independent of NF-κB/IκB transcription factors. Naunyn Schmiedeberg's Arch. Pharmacol. 361, 636–645.
54. Bourke, E., Moynagh, P. N. (1999) Antiinflammatory effects of glucocorticoids in brain cells, independent of NF-κB. J. Immunol. 163, 2113–2119.
55. Costas, M. A., Muller Igaz, L., Holsboer, F., Arzt, E. (2000) Transrepression of NF-κB is not required for glucocorticoid protection of TNF-α-induced apoptosis on fibroblasts. Biochim. Biophys. Acta 1499, 122–129.
56. Adcock, I. M., Brown, C. R., Gelder, C. M., Shirasaki, H., Peters, M. J., Barnes, P. J. (1995) Effects of glucocorticoids on transcription factor activation in human peripheral blood mononuclear cells. Am. J. Physiol. 268, C331–C338.
57. Tuckermann, J. P., Reichardt, H. M., Arribas, R., Richter, H., Schutz, G., Angel, P. (1999) The DNA binding-independent function of the glucocorticoids receptor mediates repression of AP-1-dependent genes in skin. J. Cell Biol. 147, 1365–1370.
58. Steer, J. H., Kroeger, K. M., Abraham, L. J., Joyce, D. A. (2000) Glucocorticoids suppress tumor necrosis factor-α expression by human monocytic THP-1 cells by suppressing transactivation through adjacent NF-κB and c-Jun-activating transcription factor-2 binding sites in the promoter. J. Biol. Chem. 275, 18432–18440.
59. Gonzalez, M. V., Jimenez, B., Berciano, M. T., Gonzalez-Sancho, J. M., Caelles, C., Lafarga, M., Munoz, A. (2000) Glucocorticoids antagonize AP-1 by inhibiting the activation/phosphorylation of JNK without affecting its subcellular distribution. J. Cell Biol. 150, 1199–1207.
60. Hart, L., Lim, S., Adcock, I., Barnes, P. J., Chung, K. F. (2000) Effects of inhaled corticosteroid therapy on expression and DNA-binding activity of nuclear factor κB in asthma. Am. J. Respir. Crit. Care Med. 161, 224–231.
61. Konig, H., Ponta, H., Rahmsdorf, H. J., Herrlich, P. (1992) Interference between pathway-specific transcription factors: glucocorticoids antagonize phorbol ester-induced AP-1 activity without altering AP-1 site occupation in vivo. EMBO J. 11, 2241–2246.
62. Teurich, S., Angel, P. (1995) The glucocorticoids receptor synergizes with Jun homodimers to activate AP-1-regulated promoters lacking GR binding sites. Chem. Senses 20, 251–255.
63. Bailey, S., Hall, A. G., Pearson, A. D. J., Redfern, C. P. F. (2001) The role of AP-1 in glucocorticoid resistance in leukaemia. Leukemia 15, 391–397.
64. Pearce, D., Matsui, W., Miner, J. N., Yamamoto, K. R. (1998) Glucocorticoid receptor transcriptional activity determined by spacing of receptor and nonreceptor DNA sites. J. Biol. Chem. 273, 30081–30085.
65. Diamond, M. I., Miner, J. N., Yoshinaga, S. K., Yamamoto, K. R. (1990) Transcription factor interactions: selectors of positive or negative regulation from a single DNA element. Science 249, 1266–1272.
66. Di Lorenzo, D., Williams, P., Ringold, G. (1991) Identification of two distinct nuclear factors with DNA binding activity within the glucocorticoid regulatory region of the rat alpha-1-acid glycoprotein promoter. Biochem. Biophys. Res. Commun. 176, 1326–1332.
67. Jungman, R. A., Wang, X-S., Milkowski, D. M., Short, M. L. (1992) Glucocorticoid induction of CRE-binding protein isoform mRNA in rat C6 glioma cells. Nucleic Acids Res. 20, 825–829.
68. Mittal, R., Kumar, K. U., Pater, A., Pater, M. M. (1994) Differential regulation by c-jun and c-fos protooncogenes of hormone response from composite glucocorticoid response element in human papilloma virus type 16 regulatory region. Mol. Endocrinol. 8, 1701–1708.
69. Wei, P., Vedeckis, W. V. (1997) Regulation of the glucocorticoids receptor gene by the AP-1 transcritpion factor. Endocrine 7, 303–310.
70. Keppola, T. K., Luk, D., Curran, T. (1993) Fos is a preferential target of glucocorticoids receptor inhibition of AP-1 activity in vitro. Mol. Cell. Biol. 13, 3782–3791.
71. Freedman, L. P. (1999) Increasing the complexity of coactivation in nuclear receptor signaling. Cell 97, 5–8.
72. Aarnisalo, P., Palvimo, J. J., Janne, O. A. (1998) CREB-binding protein in androgen receptor-mediated signaling. Proc. Natl. Acad. Sci. USA 95, 2122–2127.
73. Kino, T., Nordeen, S. K., Chrousos, G. P. (1999) Conditional modulation of glucocorticoids receptor activities by CREB-binding protein (CBP) and p300. J. Steroid Biochem. Mol. Biol. 70, 15–25.
74. McKay, L. I., Cidlowski, J. A. (2000) CBP (CREB binding protein) integrates NF-κB (nuclear factor-κB) and glucocorticoids receptor physical interactions and antagonism. Mol. Endocrinol. 14, 1222–1234.
75. Kurihara, I., Shibata, H., Suzuki, T., Ando, T., Kobayashi, S., Hayashi, M., Saito, I., Saruta, T. (2000) Transcriptional regulation of steroid receptor coactivator-1 (SRC-1) in glucocorticoids action. Endocr. Res. 26, 1033–1038.
76. Na, S-Y., Lee, S-K., Han, S-J., Choi, H-S., Im, S-Y., Lee, J. W. (1998) Steroid receptor coactivator-1 interacts with the p50 subunit and coactivates nuclear factor-κB-mediated transactivations. J. Biol. Chem. 273, 10831–10834.
77. Belvisi, M. G., Wicks, S. L., Battram, C. H., Bottoms, S. E., Redford, J. E., Woodman, P., Brown, T. J., Webber, S. E., Foster, M. L. (2001) Therapeutic benefit of a dissociated glucocorticoids receptor and the relevance of in vitro separation of transrepression from transactivation activity. J. Immunol. 166, 1975–1982.
78. De Bosscher, K., Vanden Berghe, W., Haegeman, G. (2001) Glucocorticoid repression of AP-1 is not mediated by competition for nuclear coactivators. Mol. Endocrinol. 15, 219–227.
79. Riccardi, C., Cifone, M. G., Migliorati, G. (2001) Glucocorticoid hormone-induced modulation of gene expression and regulation of T-cell death: role of GITR and GILZ, two dexamthasone-induced genes. Cell Death Differ. 6, 1182–1189.
80. Cannarile, L., Zollo, O., D'Adamio, F., Ayroldi, E., Marchetti, C., Tabilio, G., Bruscoli, S., Riccardi, C. (2001) Cloning, chromosomal assignment and tissue distribution of human GILZ, a glucocorticoid hormone-induced gene. Cell Death Differ. 8, 201–203.
81. D'Adamio, F., Zollo, O., Moraca, R., Ayroldi, E., Bruscoli, S., Bartoli, A., Cannarile, L., Migliorati, G., Riccardi, C. (1997) A new dexamethasone-induced gene of the leucine zipper family protects T lymphocytes from TCR/CD3-activated cell death. Immunity 7, 803–812.
82. Mittelstadt, P. R., Ashwell, J. D. (2001) Inhibition of AP-1 by the glucocorticoid-inducible protein GILZ. J. Biol. Chem. 276, 29603–29610.
83. Ayroldi, E., Migliorati, G., Bruscoli, S., Marchetti, C., Zollo, O., Cannarile, L., D'Adamio, F., Riccardi, C. (2001) Modulation of T-cell activation by the glucocorticoid-induced leucine zipper factor via inhibition of nuclear factor-κB. Blood 98, 743–753.